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SPONTANEOUS COCCIDIOIDAL GRANULOMA IN THE LUNGS OF WILD RODENTS

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Emmons [1] recently reported the finding of fungi in 25 of 105 animals trapped in the desert around San Carlos, Ariz. His investigation was undertaken because of the demonstration by Aronson, Saylor and Parr [2] that a high percentage of Indian school children on the San Carlos Indian Reservation were sensitized to coccidioidin and because the concept that Coccidioides is a soil-inhabiting fungus seemed inconsistent with difficulties experienced in isolating it from soil. Of the 25 fungus-infected animals, 7 of the pocket mice (Perognathus), 1 kangaroo rat (Dipodomys) and 1 ground squirrel (Citellus) had gross lesions in their lungs at autopsy.

The purpose of this report is to describe these lesions and the fungus associated with them.

GROSS OBSERVATIONS

On gross examination the lungs of 1 mouse showed five nodules. Two pulmonary nodules were present in each of 3 mice, and one was found in each of the remaining 3. A single nodule was found in the lungs of the kangaroo rat, and the ground squirrel had many minute nodules scattered irregularly throughout all lobes.

Although the number of animals and the total number of lesions are too small for reliable analysis as to location, it appears that the lower lobes of the lungs are most often involved. The nodules frequently were located in the wedge-shaped portion of the lung which lies between the pericardial sac and the anterior wall of the chest and in that portion of the lung which occupies the costophrenic angle.

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1. Emmons, C. W.: Pub. Health Rep. **57**:109, 1942.

2. Aronson, J. D.; Saylor, R. M., and Parr, E. I.: Relationship of Coccidioidomycosis to Calcified Pulmonary Nodules, Arch. Path. **34**:31, 1942.

Most of the nodules, excluding those in the ground squirrel, were subjacent to the pleura or superficially located, where they could be seen without gross sectioning. Only one additional nodule was found by this sectioning, which was done in approximately 1 mm. steps. This nodule was located near the hilus of a lower lobe.

Although exact measurement of the lesions was not attempted grossly, the majority appeared to be about 1 mm. in diameter. The nodules occasionally protruded slightly above the level of the surrounding lung surface and were sharply circumscribed. In the unfixed state they were semitransparent, and after fixation in a 4 per cent solution of formaldehyde they were yellowish gray to gray, which contrasted sharply with the brown of the uninvolved lung.

HISTOLOGIC OBSERVATIONS

On microscopic examination, twenty granulomatous nodules were found and examined in serial sections. One nodule which was recognized grossly as a single lesion was found to be formed of seven compactly grouped nodules, varying in size from 200 to 800 microns. Many of these were in contact with one another, but there was no confluence. Only one nodule was found microscopically which had not been seen on gross examination.

In 4 mice, seven pulmonary nodules, varying in size from 500 to 1,000 microns, were formed of fusiform epithelioid cells, which were quite irregularly disposed in the central zone, whereas at the periphery they were often arranged in very short fascicles or whorls. Two of the nodules had external layers of epithelioid cells showing concentric arrangement. These cells and their nuclei were longer and thinner than those forming the bulk of the nodules, which made this layer quite distinct. One nodule had a sharply limited external dense layer of small lymphocytes. In another a layer of such cells was present but ill defined. The cytoplasm of the epithelioid cells forming the central portion of the nodules was oxyphilic, and between some of these cells there were minimal amounts of oxyphilic granular material but no nuclear debris. A few collagen fibers coursing irregularly between the cells were present in only one of the nodules.

Two nodules, in 1 mouse, differed slightly from those just described in that they had small central areas of necrosis and the epithelioid cells generally were very thin and long. These cells were often arranged in fascicles, which tended to accentuate their length.

A variation in the type of granuloma considered in the foregoing paragraph was seen in four small lesions (300 to 400 microns) which formed a part of the conglomerate mass mentioned earlier. Peripherally there was a compact layer of concentrically arranged fusiform epithelioid cells, and centrally there was a sharply delineated sheet of large mononuclear cells having wide zones of oxyphilic granular cytoplasm. At

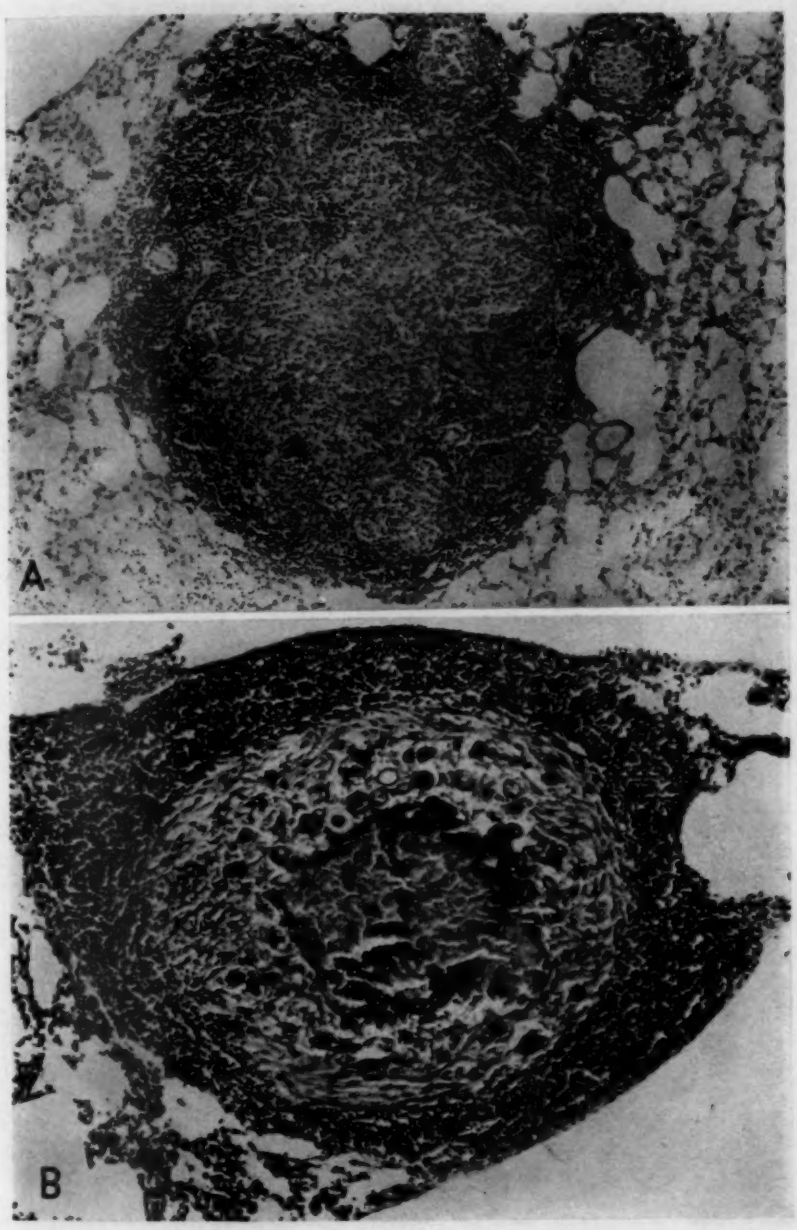

Fig. 1.—*A*, well circumscribed epithelioid cell granuloma, 1 mm. in diameter, showing a little central karyorrhectic necrosis and an external lymphocyte collar. *B*, epithelioid cell granuloma measuring 500 microns in diameter, showing central necrosis with calcification, and many variably sized fungus cells.

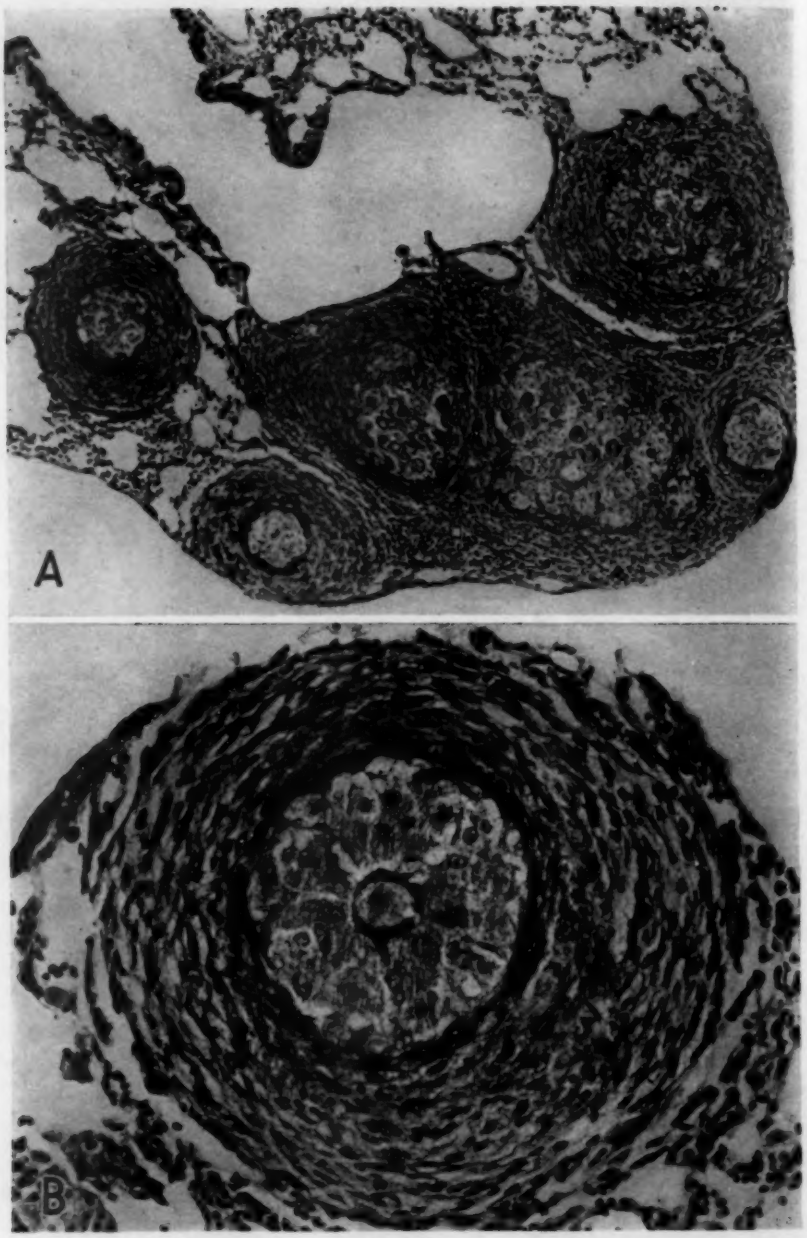

Fig. 2.—*A*, conglomerate epithelioid cell granuloma, with many fungus cells but no caseation necrosis. Note dilated bronchiole abutting on mass. *B*, higher magnification of one of the round lesions seen in *A*. Note the centrally located fungus cell surrounded by large polygonal mononuclear cells. This granuloma measured 300 microns in diameter.

the junction of the two layers there was some cell compression with early degenerative changes.

Six other nodules, occurring in 3 additional mice, were basically similar to those first described, but showed large central areas of necrosis. These nodules varied in diameter from 500 to 1,200 microns. The ratio between the size of the caseous center and the thickness of the cellular wall was quite variable. One lesion presented a necrotic center 300 microns in diameter and an epithelioid cell wall 100 microns in thickness, while another nodule had a 300 micron area of central necrosis and a cellular wall of the same thickness. The areas of necrosis were sharply limited peripherally and in general conformed to the shape of the nodules. These necrotic areas showed very little basophilic nuclear debris; when present, it often was clumped in a central or slightly eccentric location. In one lesion most of the necrotic material was calcified. Most of these granulomatous structures showed occasional to few small ill defined satellite areas of necrosis in the inner margin of the epithelioid cell wall. This wall was essentially similar to those seen in the noncaseous lesions. One had an outer circular layer of epithelioid cells and an inner layer in which the cells showed no polarity or were grouped in whorls or tufts, while in four the outer circular layer was absent. Distinct palisading was not seen in any of the nodules, but in three there were a few fusiform epithelioid cells radially arranged in the peripheral margin of the caseous material. The outer margins of these nodules were not as sharp as those of lesions showing little or no central necrosis. In the outer part of the walls of these, the epithelioid cells were often short and plump, and there were present from few to many large mononuclear cells. A few of the latter were seen in mitosis, and many contained nuclear debris. The recognizable alveoli adjacent to the nodules usually showed some enlargement of the septal cells. One of the centrally caseous nodules had a distinct, densely cellular lymphocyte mantle measuring 50 microns in thickness.

Five of the 11 lesions thus far described, excluding the satellite lesions seen in 1 mouse, were both parabronchial and subpleural in location. Three were subpleural but not related to bronchi; one parabronchial nodule was deep in the pulmonary parenchyma, and two were related neither to the pleura nor to the bronchi. The pleura overlying the superficial nodules often was elevated slightly, showed fibroblast proliferation, and infiltration by a few lymphocytes and macrophages. A few of the nodules had produced pressure distortion of the adjacent bronchi, and one had destroyed part of a bronchial wall and there was proliferation of epithelioid cells into its lumen.

An additional nodule, measuring 1 mm. in diameter and located adjacent to a bronchiole, was formed mainly of short fusiform epithelioid cells which were proliferating within alveoli. Occasionally these cells were arranged in fairly distinct whorls, which accentuated the com-

pressed but generally recognizable alveolar septums. In an eccentric location there was an area of karyorrhectic necrosis. This area radiated to the periphery, where it involved the wall of the marginating bronchiole. From the granuloma, epithelioid cells proliferated into and partly filled the lumen of the bronchiole.

One subpleural cellular lesion, measuring 600 by 800 microns, was not of the fusiform epithelioid cell type. It was formed of large coherent mononuclear cells, which filled most alveoli in the involved area. Between groups of such cells, particularly in the peripheral part of the lesion, there were interrupted strands of lymphocytes and fewer neutrophils.

Pulmonary lesions in the ground squirrel were numerous. They varied from 30 or 40 microns to 1 mm. in diameter, but most were less than 500 microns. All of these lesions were of the large mononuclear cell type just described but showed a slightly greater prominence of lymphoid cells.

The one nodule found in the kangaroo rat was both subpleural and parabronchial and measured 1 mm. in diameter. Its wall was essentially similar to that of the noncaseous nodules in the mice, but the central portion was formed of large mononuclear cells and showed very slight focal necrosis. The epithelioid cell wall showed a moderate amount of focally hyalinized fibrous tissue. A sharply limited dense layer of lymphocytes, 200 microns in thickness, surrounded the nodule.

FUNGI PRESENT IN THE GRANULOMATOUS NODULES

Fungi were present in all of the lesions. They varied in number from three or four to fifty in single sections, and in general they were more numerous in the noncaseating nodules. They varied in size from 4 to 50 microns in diameter, but those measuring from 20 to 30 microns were infrequent, and larger forms were rare. In the noncaseous lesions they were fairly evenly distributed throughout the central two thirds, while in the caseous ones they were mainly limited to the inner half of the epithelioid cell wall. Only a few were present in the areas of necrosis, and these usually were small. It was mentioned in an earlier paragraph that epithelioid cells often were arranged in small whorls and that satellite areas of necrosis often were present in the inner part of the cellular wall. One or more fungus cells usually could be demonstrated in such areas. Proliferation of epithelioid and large mononuclear cells around centrally located single fungus cells was distinct in two small nodules.

The fungus cells had doubly contoured walls and variable amounts of lightly basophilic, irregularly and finely vacuolated cytoplasm. As they increased in size, the amount of cytoplasm became relatively smaller in amount; often it formed only a thin layer on the inner surface

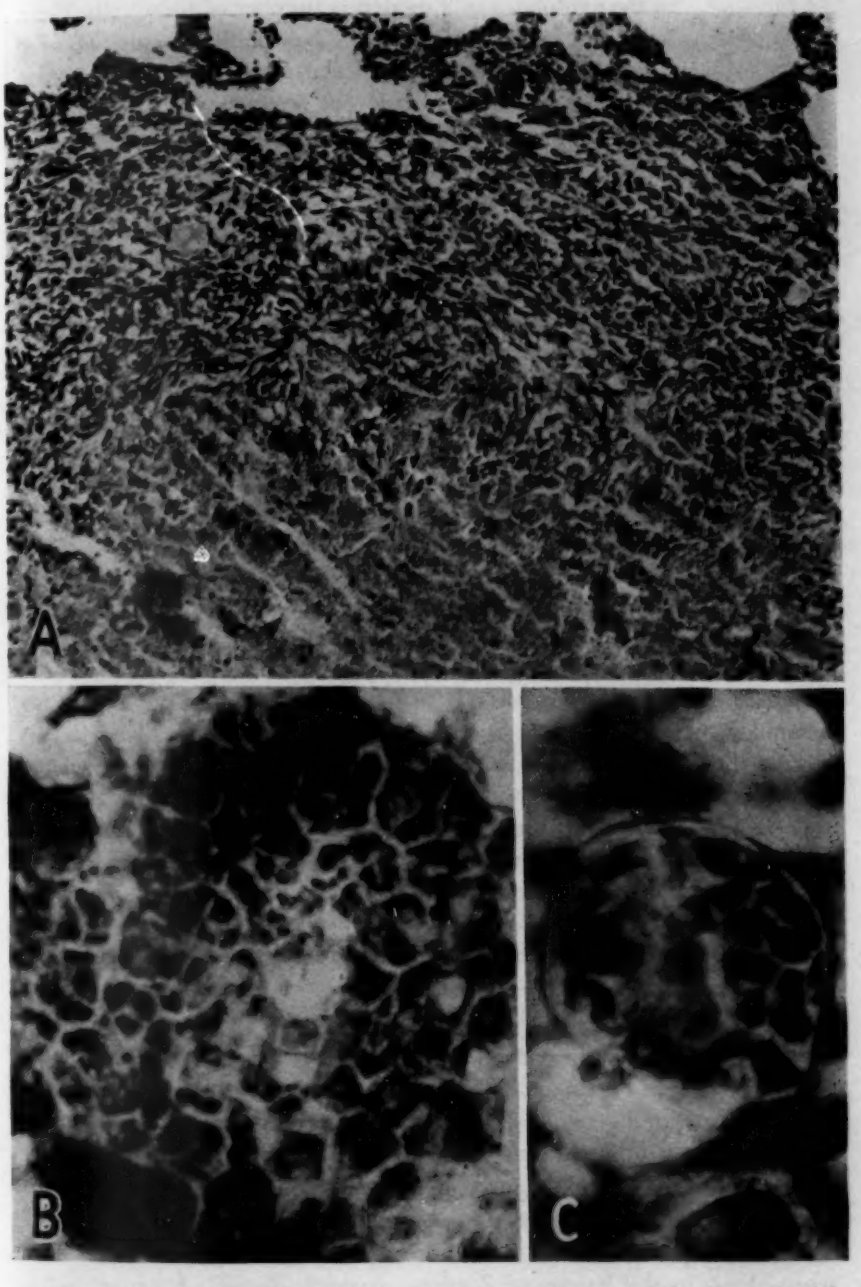

Fig. 3.—*A*, portion of a centrally caseous granuloma; × 142. *B*, tangential section of a spore showing cytoplasmic cleavage and endospore formation; the sporangial wall is not shown; × 1369. *C*, sporangium with endospores, almost "ripe"; × 1568.

of the wall. In the cytoplasm of a moderate number of these cells, there were present small but fairly distinct deeply basophilic granules. These granules were not numerous, nor were they evenly spaced, but their distribution pattern was such as to rule out irregularity of staining as being responsible for them.

In two nodules from 2 pocket mice there were six fungus cells which had reached that stage of maturity where they assume the reproductive functions of sporangia. They were from 23 to 50 microns in size and showed distinct endosporulation. The progressive cytoplasmic cleavage and the shape of the endospores were typical of the developing Coccidioides spherules seen in human and experimental infections. The endospores formed a peripheral layer beneath the doubly contoured wall and surrounded a large central vacuole. One sporangium was ripe and completely filled with small spores, the central vacuole being absent. Partially collapsed sporangia containing nuclear debris but no spores were found in the necrotic zones of three nodules from the 2 mice mentioned earlier and also in a fourth nodule from another animal.

In addition to the intragranulomatous fungi described, few to many were also found scattered throughout the lungs. These fungus cells, although quite similar, showed some differences in appearance and staining reaction from those within the lesions. These fungi were found in all of the animals discussed here with the exception of the kangaroo rat. They were seen also in 8 of the 13 additional animals examined, which showed no granulomatous involvement. The relationship of these forms to those within the nodules and to the fungi isolated in cultures from these animals is still under study. The 3 animals reported by Emmons [1] from which Coccidioides immitis was cultured showed no gross lesions. Lungs from these animals, which were trapped early in the study and before it became apparent that a prevalent mycosis was present in the rodents, were not saved.

Grossly all organs other than the lungs were normal. The liver, the kidney and the spleen were examined microscopically in 3 cases, and the pancreas, in 4. They showed no significant alterations.

COMMENT

The etiologic agent in all of the lesions was a nonbudding fungus cell having centrally vacuolated basophilic cytoplasm and a doubly contoured wall. To identify such fungus cells as the parasitic phase of Coccidioides, it is necessary to demonstrate progressive cytoplasmic cleavage and the formation of endospores. Such endosporulating forms were found in the lesions of 2 mice. In view of the similarity of the lesions and the presence of developing fungi in all, we feel justified in describing the lesions as coccidioidal granulomatous nodules.

These lesions occurring in wild rodents in spontaneous Coccidioides infection are of proliferative rather than exudative type. This in addition to the caseous necrosis which is often present suggests slow formation and growth. In experimental Coccidioides infection, cell infiltration is a prominent feature, and neutrophils form a large part of the exudate. This reaction was not present in any of the granulomatous lesions found in the spontaneous infection.

The fact that in the material examined so few sporangia were mature and filled with endospores suggests that the host animal, at least during the season it was collected, had an inhibiting influence on this maturation cycle. The ratio of spore size to the amount of cytoplasm is also in keeping with this concept, in that growth of the cytoplasm does not keep pace with the increasing spore size.

If one excepts the seven nodules seen as one in 1 animal, there remain thirteen nodules, ten of which were of remarkably uniform size. Nine measured 1 mm. in diameter, and one measured 1,200 microns. Since it seems reasonable to assume that the various animals were not infected at the same time, the uniform size of these nodules suggests that they were chronic lesions whose rate of growth decreased with increasing age. The animal excepted from the aforementioned group is the one which showed seven closely grouped nodules of variable size. It is believed that this is the only instance in this series in which dissemination took place from the primary lesion, with the formation of satellite nodules.

It is impossible from this material to determine the fate of the granulomatous nodules. The answer to this question has an important bearing on the epidemiology of the disease, a phase which needs much clarification. However, it appears from the nature of the lesions that rapid extension to other organs and tissues, such as occurs in experimental infection, is not to be expected. The chronic process found is what one would expect in an effective animal reservoir.

SUMMARY

Of 105 rodents trapped in the desert around San Carlos, Ariz., 9 showed gross pulmonary lesions—7 pocket mice (Perognathus), 1 kangaroo rat (Dipodomys) and 1 ground squirrel (Citellus). Microscopically, a total of twenty nodular lesions were found in the 7 mice and 1 kangaroo rat. The lesions occurred most often in the lower lobes of the lungs and particularly along the anterior border and in that portion of the lung occupying the costophrenic angle. Most of them were superficially located, and many caused slight elevation of the overlying pleura. Except for those in 1 animal (discussed in the text), most of the nodules measured 1 mm. in diameter. Basically each

was formed of fusiform epithelioid cells, diffusely and irregularly disposed in the center but often showing concentric arrangement peripherally. A few lesions were formed centrally of adherent large mononuclear cells. Six nodules had large central zones of caseous necrosis, varying from 300 to 800 microns in diameter; one of these was partially calcified. Fibrosis was present in only two lesions, being of moderate degree in one and slight in the other. A few lymphocytes were present in the outer wall of most nodules, and three had sharply limited, densely cellular lymphocyte mantles, varying from 25 to 200 microns in thickness. The multiple small pulmonary lesions present in the ground squirrel were formed of grouped alveoli filled with sheets of large mononuclear cells. In all of the nodules there were present few to many fungus cells of varying size. In two nodules (2 mice) six fungi showed cytoplasmic cleavage or mature endospores typical of Coccidioides.

REDUCTION OF PULMONARY RESISTANCE TO INFECTION BY CIRCULATING TOXINS

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DURHAM, N. C.

The onset of many types of pneumonia is generally preceded by a lowering of the resistance of the body as a whole or of the respiratory tract in particular. The part played by viral diseases in decreasing the resistance of the respiratory tract to secondary infection is well recognized. Since it has been shown [1] that certain bacterial toxins are capable of damaging the lungs in a similar manner, we thought it possible that toxins reaching the lungs by way of the blood stream might cause sufficient injury to allow the entrance of bacteria.

In this paper we report certain experiments which demonstrate the ability of toxins injected intravenously to damage the lungs and show that similar damage may occur in man when the toxin enters the circulation from a focus of infection.

METHODS AND MATERIALS

Animals.—Adult rabbits weighing about 2 Kg. were used.

Toxin.—Staphylococcus toxin similar to that described in a previous paper [1] was employed in these experiments.

Mode of Injection.—One part of the toxin was diluted with 4 parts of physiologic solution of sodium chloride and injected slowly into an ear vein of each rabbit.

Necropsy.—Each animal was killed by a sharp blow at the base of the skull and a necropsy made immediately. The thorax was opened aseptically, and portions of the lungs were cultured on blood agar plates and both aerobically and anaerobically in broth. All animals whose cultures did not remain sterile were excluded from the experiment. The lungs were then removed, inflated and fixed in Zenker's solution. They were later sectioned and embedded in paraffin and microscopic preparations made. These were stained with hematoxylin and eosin and for bacteria.

EXPERIMENTAL STUDY

Thirty-two rabbits were given intravenous injections of staphylococcus toxin. Sixteen of these received a sublethal dose (1 cc.). These animals were killed one, two, three, four and five days after inoculation.

From the Department of Pathology of Duke University School of Medicine.

1. Sprunt, D. H.; Martin, D. S., and Williams, J. E.: J. Exper. Med. **62**:73, 1935.

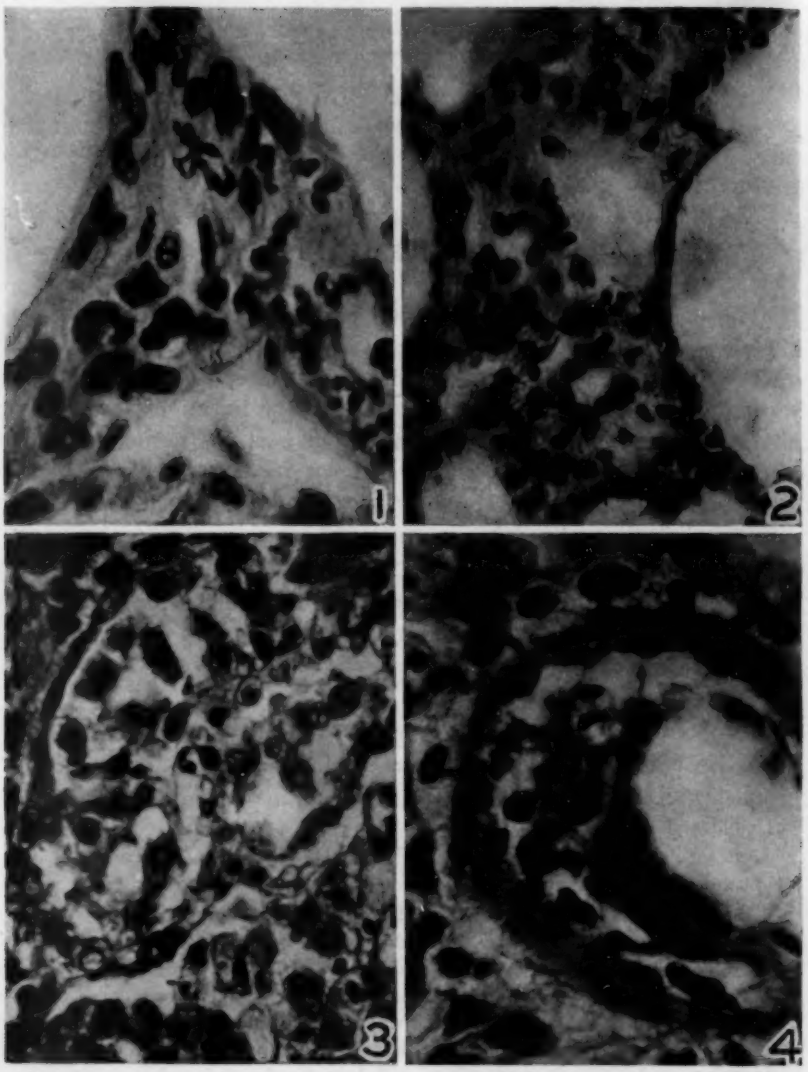

Fig. 1.—Section of a lung of a rabbit killed seventy-two hours after an injection of staphylococcus toxin, showing proliferation of cells in the interstitial tissue. Hematoxylin and eosin; × 784.

Fig. 2.—Another section illustrating the same changes as were shown in figure 1. Hematoxylin and eosin; × 784.

Fig. 3.—Section of a lung of a rabbit killed seventy-two hours after an injection of staphylococcus toxin, showing macrophages in an alveolus. Hematoxylin and eosin; × 784.

Fig. 4.—Section of a lung from a rabbit killed seventy-two hours after an injection of toxin, showing proliferation of the intima of a blood vessel. Hematoxylin and eosin; × 784.

The other 16 were given a large amount (2 cc.). Eight of these died in less than twenty-four hours, and the others were killed at the end of this period. As controls, the lungs of 9 rabbits given injections of

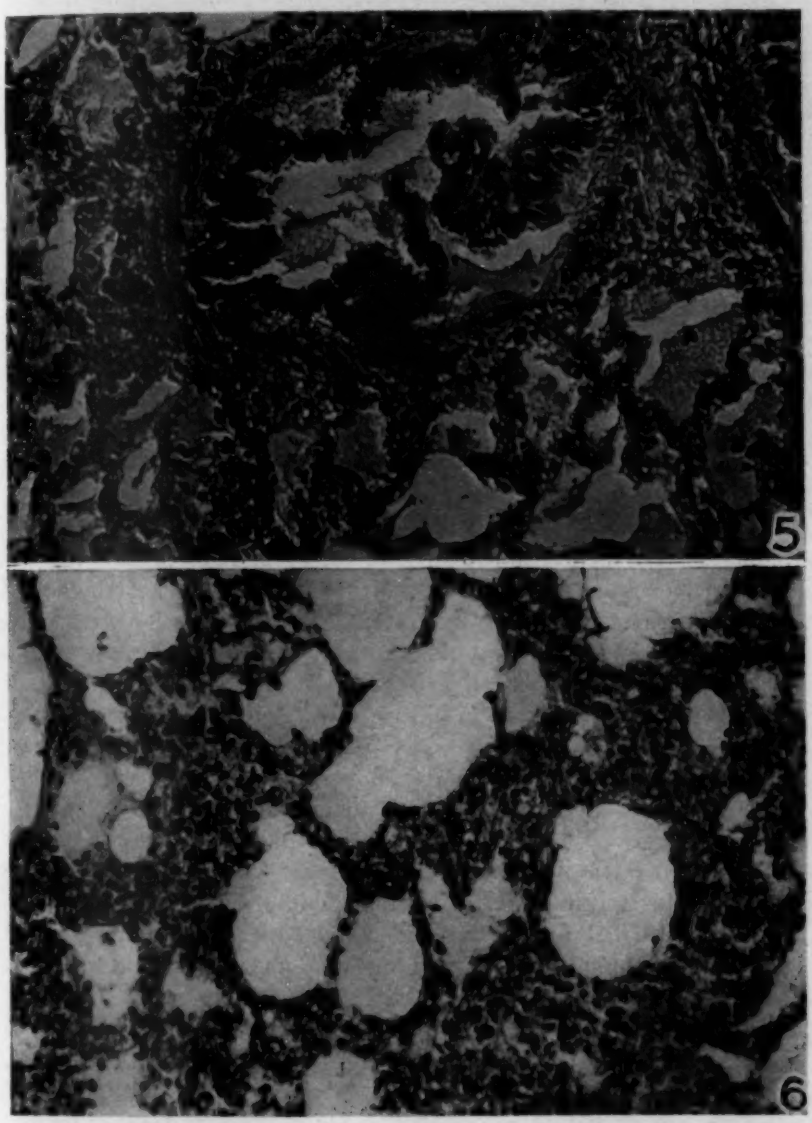

Fig. 5.—Section from a lung of a child with acute mastoiditis, showing extensive edema of the lung and separation of the epithelium from the basement membrane of the lung. Hematoxylin and eosin; × 179.

Fig. 6.—Section from a lung of a child with otitis media, showing marked proliferation of interstitial tissue. Hematoxylin and eosin; × 179.

heated toxin and also the lungs from a number of normal rabbits were studied.

At necropsy the lungs showed no gross lesions. The microscopic preparations from all the experimental rabbits showed diffuse lesions. There was considerable variation between the individual rabbits. No changes of any significance were seen in the controls.

Three animals which died in less than thirty minutes showed extensive edema and some hemorrhage. Numerous cells were seen in the interstitial tissue and particularly in the perivascular lymphatics. Although large numbers of these were polymorphonuclear leukocytes, a number of mononuclear cells were also present. The bronchi and bronchioles all contained red blood cells. The animals which died between one and twenty-four hours and those killed at this time showed various degrees of change. In some animals, hemorrhage was the predominant feature; in others, cellular infiltration of the interstitial tissue was the most prominent change. The infiltrate comprised both polymorphonuclear and mononuclear cells. In addition to the findings mentioned some areas were observed in which the alveoli were filled with mononuclear cells (figs. 1, 2 and 3).

In addition to these changes there was another lesion which, although relatively infrequent, was thought to be of particular interest. This lesion consisted of an invasion of the basal layer of the bronchial and bronchiolar epithelium with cells and a separation of these cells from the basement membrane by edema. This lesion is shown in figure 6. Such a lesion as this would obviously provide a fertile soil for entrance of a secondary infection to the bronchial tree.

After forty-eight hours the picture was essentially the same except that a few more mononuclear cells were present in the perivascular lymphatics and also in the interstitial tissue. One interesting finding at this time and later was an occasional proliferation of the intima of the blood vessels as shown in figure 4. At the end of seventy-two hours the perivascular lymphatics were filled with mononuclear cells and the interstitial tissue contained focal accumulations of mononuclear cells. The animals killed more than three days after inoculation showed essentially the same thing. In 1 instance a small area of necrosis was found.

NECROPSY OBSERVATIONS ON MAN

Since the experiments just described had shown that the intravenous injection may cause pulmonary damage, it was thought likely that similar changes might be found in human cases of bacterial toxemia. A survey of 1,600 necropsies revealed many instances in which changes similar to those described in the experimental animals were found. In a previous paper,[2] however, it was shown that this type of reaction can

2. Sprunt, D. H.: South. M. J. **31**:362, 1938.

also be caused by viruses, certain bacteria, resolving pneumonia, lipoids, hemosiderin and other foreign substances. Therefore, it was thought essential to rule out all of these as factors in the production of this reaction before we could attribute the change to bacterial toxins. To do this the records of all the cases were studied for any history of a viral or a bacterial infection of the lung or a resolving bacterial pneumonia. Microscopic preparations of the lung also were examined for the presence of inclusion bodies, bacteria, lipoid, hemosiderin or foreign material which might have caused this pulmonary reaction. The application of these criteria led to the exclusion of all but 5 cases. In these 5, however, no other cause could be found; hence, we felt justified in attributing the pulmonary lesions to a toxemia. The foci from which the toxin reached the lungs were foci of puerperal infection, peritonitis, mastoiditis with meningitis and, in 2 instances, otitis media. The changes produced in these lungs were similar to those described in the animals and are shown in figures 5 and 6.

It is thought likely that some of the cases which were excluded because of the presence of bacteria in the lungs were actually secondary infections following damage to the lungs by circulating toxins.

COMMENT

Our experiments showed that the lungs may be damaged by a toxin injected intravenously. This observation was further substantiated by a study of necropsy records for human beings and microscopic slides which showed that the lungs could be damaged in a similar manner by the dissemination of toxin into the blood stream from a distant focus. In the experiments it was demonstrated that the type of reaction could be controlled by the amount of toxin injected, a small amount giving a mononuclear type of reaction and a larger amount an edematous and hemorrhagic reaction.

The type of reaction produced was similar to that caused by the intratracheal injection of toxin except that it was much more diffuse. This was to be expected, as the toxin when injected into the trachea tends to damage only the portion of the lung to which it gains access, whereas when it is injected intravenously it becomes well mixed with the blood in the right ventricle and is diffused throughout the whole lung.

These observations are important as they show that pulmonary damage may occur as the result of toxemia from a distant focus even in the absence of actual bacteremia. The pulmonary damage, while in itself not extensive, may lead to lowering of the local resistance of the lung and thus predispose to secondary bacterial invasion and resultant pneumonia.

SUMMARY

Experiments are reported in which it was demonstrated that the intravenous injection of staphylococcus toxin damages the parenchyma of the lungs, the bronchi and the bronchioles. This damage may reduce the lung resistance so that a secondary bacterial infection may occur.

Similar changes are reported as occurring in lungs of man in cases in which there is a focus of infection from which toxins may reach the lung.

ELASTIC TISSUE

I. DESCRIPTION OF A METHOD FOR THE ISOLATION OF ELASTIC TISSUE

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Several physical and morphologic studies have been made in an effort to determine the changes which occur in elastic tissue in numerous diseases.[1] The interpretation of the data has depended on the assumption that the measurements of the elasticity of the tissues are a reliable indication of the properties of the elastic networks. Inasmuch as all studies have been made on complex tissues, the correctness of this assumption may be questioned. Hence, it is desirable to undertake a simultaneous physical, morphologic and chemical study of purified elastic networks. The first step in this study is the development of a method which not only assures reasonable chemical purity of the networks but also preserves a high order of elasticity, tensile strength and integrated microscopic structure. These requirements cannot be conveniently met by recourse to available methods for the isolation of elastic tissue.[2] The method to be described fulfils the requirements, at least when the object of study is the human aorta.

METHODS

The outline of such a study of the elastic tissue of the human aorta is as follows: First, determinations of the appearance, extensibility and retractility of the intact vascular wall are made. Second, the elastic networks are isolated by removal of all other components of the vascular wall. Third, studies of the appearance, extensibility, retractility and tensile strength of the purified elastic networks are made. Finally, an investigation of the factors which determine normal and abnormal qualities of elastic networks in their native and isolated states can be undertaken. The standardization of a method for the isolation of elastic networks is the first requirement in the projected study.

Method for the Isolation of Elastic Tissue.—The object of study is the fresh human aorta obtained post mortem. The segment of the vessel between the levels

From the Department of Pathology of Cornell College of Medicine.

1. Hass, G.: Arch. Path. **27**:344, 1939. Saxton, J. A.: ibid. **34**:262, 1942. Wilens, S. L.: Am. J. Path. **13**:811, 1937. Winternitz, M. C.; Thomas, R. M., and Le Compte, P. M.: Am. Heart J. **14**:399, 1937.

2. (*a*) Richards, A. N., and Gies, W. J.: J. Physiol. **7**:93, 1902. (*b*) Stein, W. H., and Miller, E. G.: J. Biol. Chem. **125**:599, 1938. (*c*) Lowry, O. H.; Gilligan, D. R., and Katersky, E. M.: ibid. **139**:795, 1941.

of the left subclavian and the twelfth intercostal arteries is resected for study. This cylindric segment is chosen for two reasons. First, it is so constructed that preparations of several cylindric rings of similar dimensions can be made. Second, if the aorta is diseased, this segment usually has morphologic changes which are an average of those of the entire vessel. After resection of the thoracic segment, the plane of cleavage at the junction of the media and adventitia is sought for. Excess adventitial connective tissue is removed by dissection along the plane. The cylindric specimen is then divided transversely at the level of the second intercostal artery. The proximal segment is used for the determination of the quantity of elastic tissue in the aortic wall. The distal segment is divided into a series of rings of equal height for physical and morphologic studies.

The quantity of elastic tissue in the proximal segment at different stages of purification is determined as follows: The segment is extracted for forty-eight hours successively with alcohol and ether and then dried to constant weight at 110 C. After weighing, the segment is rehydrated and immersed in a volume of 89 per cent formic acid, approximating 1 cc. for each 5 mg. of tissue. The flask is sealed and placed in an oven at 45 C. After twenty-four hours, the tissue is removed from the formic acid, washed to neutrality and, after dehydration with alcohol and ether, is dried to constant weight. This procedure of extraction and determination of loss of weight is repeated routinely so that values for extraction intervals of twenty-four, forty-eight and seventy-two hours are obtained. In several instances, the extraction is continued for periods up to ninety-six, one hundred and twenty, one hundred and forty-four and one hundred and sixty-eight hours. Curves which illustrate the rate of purification and probable rate of solution of elastic tissue are plotted from these data.

The distal segment of the thoracic aorta is used for physical and morphologic studies. It is divided transversely at 4 to 5 mm. intervals into a series of similar cylindric rings. Appropriate measurements of extensibility and retractility of each ring are made. Two representative rings are fixed at once for microscopic study. The remaining rings with similar physical properties are extracted with suitable volumes of 89 per cent formic acid at 45 C. After twenty-four hours, two rings are removed from the solvent and washed to neutrality. One is prepared for microscopic study. The other is used for measurements of extensibility, retractility and tensile strength. This procedure is repeated on additional rings at the end of total extraction periods of forty-eight and seventy-two hours. The physical and morphologic data obtained by study of rings at the three successive stages of purification are available for comparison with the data concerned with weight losses in the same extraction intervals. Details of all methods need not be presented at this time.

QUALITATIVE ASPECTS OF THE ACTION OF FORMIC ACID ON THE AORTA

The action of formic acid on the fresh aorta is the same as its effect on the aorta which has been dried with alcohol, ether and heat before extraction with the acid. The collagen swells and becomes gelatinous and transparent. There is an increase in all dimensions of the vascular wall.

The initial swelling of collagen is very prominent. Doubtless, this is one factor which leads to a general increase in the dimensions of the vascular wall. But this is not the only factor, because the principal

fraction of all increments persists after the collagen has been dissolved and continues to persist when the residue of the vascular wall is composed only of elastic tissue. The one necessary condition for return of the vascular wall to approximately original dimensions is neutralization of the tissue. This increase in dimensions of elastic lamellas at zero mechanical load under the influence of a chemical force is of particular interest because the elongation, at least, is nearly as great as that realizable by application of mechanical force. Likewise, the retraction of chemically extended networks on removal of the chemical force is as complete as the retraction of mechanically extended networks on removal of the mechanical force. The reversible active response of an intact supposedly unreactive tissue to a change in the composition of environmental fluids deserves further study because it may be a factor in the regulation of vascular tone.

With continued extraction there is a gradual decrease in the bulk of the vascular segment. This is due to degradation and solution of the several components of the vascular wall. The true decrease in bulk, however, is not appreciated until the residue is neutralized and dried.

A third qualitative change accompanying treatment with formic acid is a decrease in the rigidity of the vascular wall. This is especially noticeable when there is initially a large amount of collagen in the intima and media. After extraction of the collagen and of the calcium if any is present, the vascular wall becomes pliable and flaccid, although it retains a normal cylindric shape.

The decrease in rigidity during purification of the elastic networks is accompanied by an increase in the elasticity and a decrease in the tensile strength of the vascular segment. The pure networks respond to manual manipulation in a manner reminiscent of the response of a rubber band of high elasticity and low tensile strength. The elasticity remains reasonably constant in the twenty-four to ninety-six hour interval of purification. The tensile strength progressively diminishes in this interval. The rate of decrease is somewhat greater than the probable rate of solution of the elastic tissue. Hence, physical measurements should be made on tissues which have had a standard period of controlled treatment.

Aged, atherosclerotic aortas occasionally disintegrate during treatment with formic acid. The disintegration occurs along a line of cleavage between the media and the thick, fibrous intima. Apparently, as collagen dissolves, the few tenuous communications between the elastic networks of the media and those of the thickened intima rupture, so that the intima splits away from the media. As a rule, however, this cleavage is incomplete and of little consequence from the standpoint of physical measurements.

The normal color of intact aortic walls varies from white to yellowish brown. The yellow tinge is ascribed to pigments dissolved in the

lipid deposits and to an intrinsic shade of color possessed by elastic tissue. During extraction the lipids and their pigments are dissolved. The color of the elastic tissue is not changed. The purified tissue of young aortas is white, while that of old aortas displays shades of color varying from light yellow to brown.

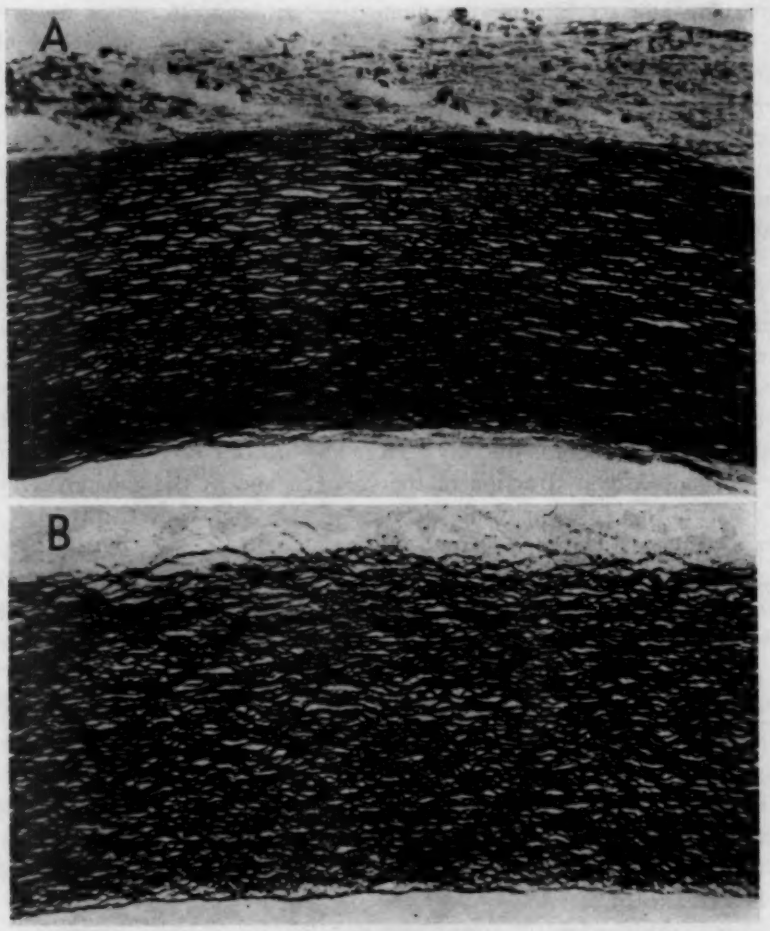

Fig. 1.—*A*, photomicrograph showing an intact aortic wall, including the adventitia. The section is stained by the Weigert-Van Gieson method for demonstrating elastic tissue. The photograph is to be compared with *B*.

B, photomicrograph of an aortic ring contiguous to the one illustrated in *A*. This ring was extracted for seventy-two hours with 89 per cent formic acid at 45 C., prior to microscopic study. Note the excellent preservation of elastic tissue in the intima, media and adventitia. The other elements of the aortic wall, best illustrated by adventitial collagen in *A*, have been extracted, with a loss of weight of 68.2 per cent of the aortic wall.

MICROSCOPIC CHANGES IN THE AORTA DURING EXTRACTION WITH FORMIC ACID

The wall of a normal young aorta is composed of endothelial cells, fibrocytes, smooth muscle cells, collagenous tissue and elastic tissue. With increasing age, macrophages, lipid deposits and calcium deposits usually appear in the aortic wall. The conditions of extraction with formic acid are such that all components of the wall are dissolved in the course of time. The utility of the present method for segregating elastic tissue depends on the variable rates of solution of the different components.

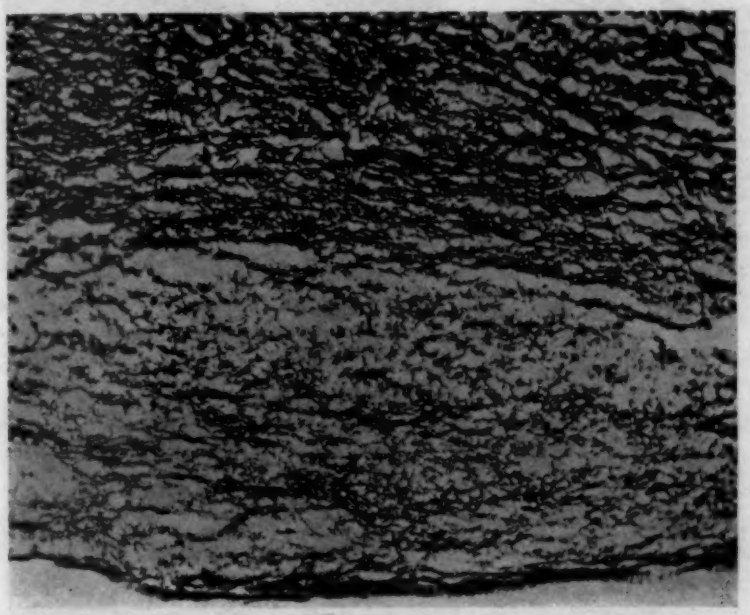

Fig. 2.—This illustration shows an intimal plaque which in the intact aorta was composed principally of dense bundles of collagen. On removal of the collagen by extraction, the residual structure of the sclerotic plaque persists in the form of a system of delicate elastic networks as shown.

Calcium salts in the dispersed form in which they occur in most aged aortas are dissolved by the reagent within one hour.

Endothelial cells, fibrocytes, smooth muscle cells and macrophages are absent in all microscopic preparations after about twelve hours' extraction.

Lipids are removed rapidly, and though no specific analyses have been made, the impression is gained that all lipids have been dissolved at the end of seventy-two hours' extraction. Most lipids are highly soluble in 89 per cent formic acid. Those of lower solubility are con-

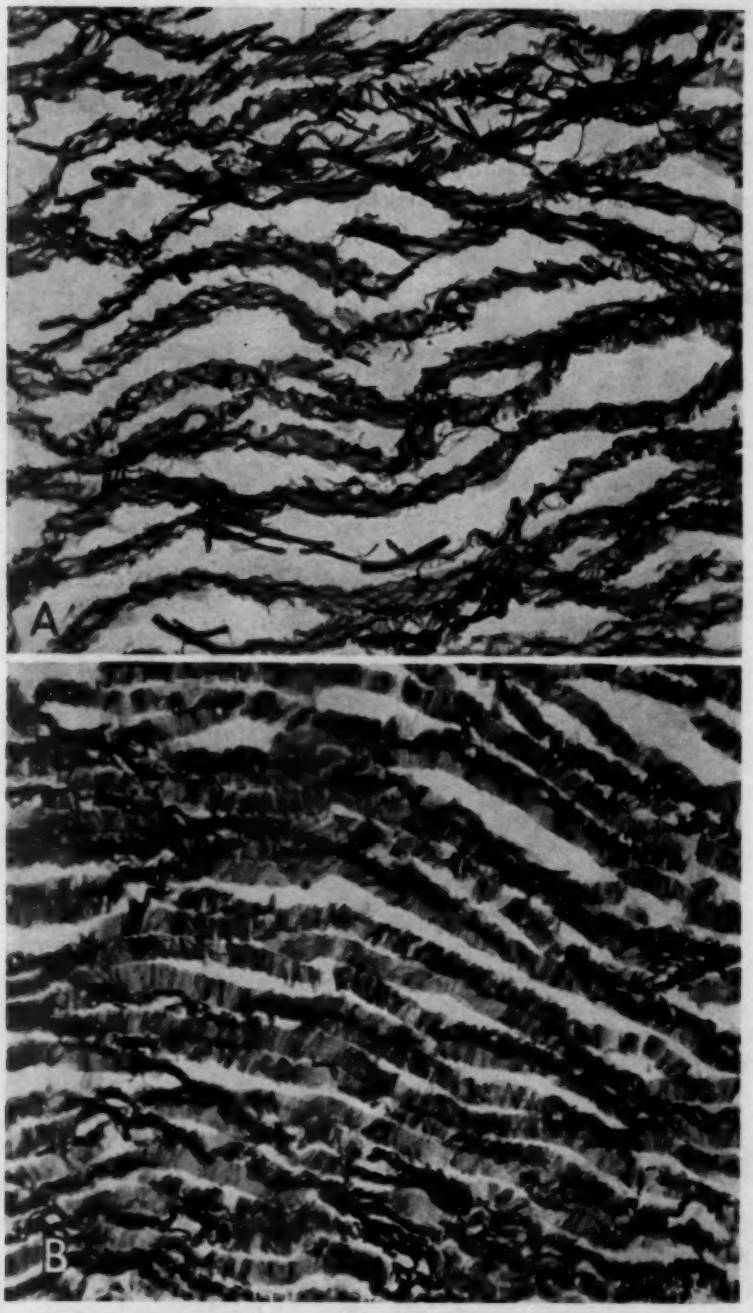

Figure 3

(See legend on opposite page)

verted to products of higher solubility by the hydrolytic action of the reagent. If necessary for special purposes, the residual lipids may be extracted with alcohol and ether, as these solvents, though they modify the elasticity of the intact vessel, have no effect on the physical or morphologic properties of purified elastic networks.

Collagen persists in the aortic wall for a longer time than other nonelastic structures. The persistence varies with the dimensions of the specimen and the compactness of its structure. In the case of delicate aortas of infants, all collagen is dissolved within twenty-four hours. In the case of thick, compact, fibrous aortas of old people, at least forty-eight to seventy-two hours of extraction is necessary for removal of collagen. It is possible that even longer periods of time may be required in some instances. So far as microscopic studies are concerned, no collagen is demonstrable in any aorta after forty-eight hours' extraction. Quantitative gravimetric studies, however, prove clearly that a considerable amount of collagen remains in the wall of some aortas after forty-eight hours' extraction. This discrepancy between morphologic and gravimetric data was settled in the first few experiments by controlling the extraction with blocks of cartilage and tendon. These collagenous tissues were degraded and dissolved at a rate comparable with the quantitative rather than with the microscopic observations.

The microscopic study of all vascular segments extracted for seventy-two hours reveals nothing except elastic tissue (figs. 1 to 3). The affinity of the tissue for the Weigert stain is fully retained even after prolonged extraction for one hundred sixty-eight hours. The delicate fibrils are easily visualized as a continuous network. An illustration of the preservation of these minute structures in a sclerotic plaque of the thickened intima of an adult aorta is shown in figure 2. Ordinarily, the delicate networks in this location are normally obscure because of dense bundles of collagen. After removal of collagen, they become sharply defined, as shown in the photograph. There is a similar exposure of fine fibrils in the media.

EXPLANATION OF FIGURE 3

A, photomicrograph showing the elastic lamellas of the aorta of a newborn infant weighing 1,300 Gm. All nonelastic elements of the media have been extracted, exposing the angulated, coiled networks from which the natural, apparently solid medial membranes are constructed.

B, elastic lamellas of the media of an adult aorta. The interlamellar cells and collagen have been removed by extraction. Though extraction does not ordinarily disclose the type of structure shown in this photograph, the peculiar coiled compact angulated systems of the lamellas shown here are similar to the elastic systems of infantile lamellas shown in *A*.

Other structural variations become evident on microscopic study of purified networks. Three common variations may be mentioned. The continuous dense elastic lamellas of the media of aortas of immature infants are not the solid cylinders that they appear to be. They are composed of angulated, interwoven networks of discrete elastic fibers. This developmental arrangement as revealed by extraction is shown in figure 3 *A*. Similar medial membranes of adult aortas often have, after extension and extraction, a corresponding though more compact, angulated or tightly coiled system, such as that shown in figure 3 *B*. A similar structure is encountered in studies of subendothelial elastic membranes. In general, however, a careful study will be necessary before the minute internal structure of seemingly homogenous adult elastic membranes can be exposed in all of its variations.

QUANTITATIVE ASPECTS OF THE ACTION OF FORMIC ACID ON THE AORTA

During extraction of all nonelastic elements from the aortic wall, elastic tissue is not wholly spared. The rate at which this tissue is degraded depends on the concentration of formic acid, the quantity of the reagent, the quantity of tissue and the temperature. For example, 1 cc. of 89 per cent formic acid dissolves several milligrams of elastic tissue in a few hours at 100 C. If the reagent contains 20 per cent formic acid, elastic tissue is resistant to its action at 100 C. If extraction is made with 89 per cent formic acid at 5 C., elastic tissue is unaffected. The segregation of the tissue at low temperature would be desirable, but all attempts failed because of persistence of nonelastic components. The search for optimum conditions disclosed circumstances which afforded a practical balance between minimal action of the reagent on elastic tissue and maximal action on other components of the aortic wall. When aortas are objects of study, 45 C. is an optimum temperature. At least 1 cc. of reagent should be used for extraction of each 5 mg. of dry tissue. The concentration of formic acid as the active reagent should not fall below 80 per cent. The time of extraction should be controlled, and a standard period should be adhered to, even though in special cases the elastic tissue may have variable impurities at the end of the standard period. There are two reasons for exercising caution in this regard. First, with each additional hour of extraction some elastic tissue, however minute the quantity, is dissolved. Second, as the quantity of elastic tissue is depleted, the tensile strength and the extensibility of the networks change in response to a standard applied weight. Under ideal conditions this would not be important since a linear relationship between depletion of elastic tissue, decrease in tensile strength and increase in extensibility under force of a standard load should exist. Under practical conditions the linear predicted relationships often do not exist.

The rate of solution of elastic tissue is not negligible but it is tolerable and probably nearly constant. This rate computed from data in the accompanying table represents a loss of 2 to 5 per cent of the available elastic tissue during each twenty-four hour period of extraction. This corresponds to an average loss of less than 2 per cent of the initial weight of the aortic wall during each twenty-four hour period. During the first forty-eight hours of extraction this loss is obscured by large simultaneous losses of nonelastic components, as illustrated in figure 4. In the forty-eight to seventy-two hour period of extraction

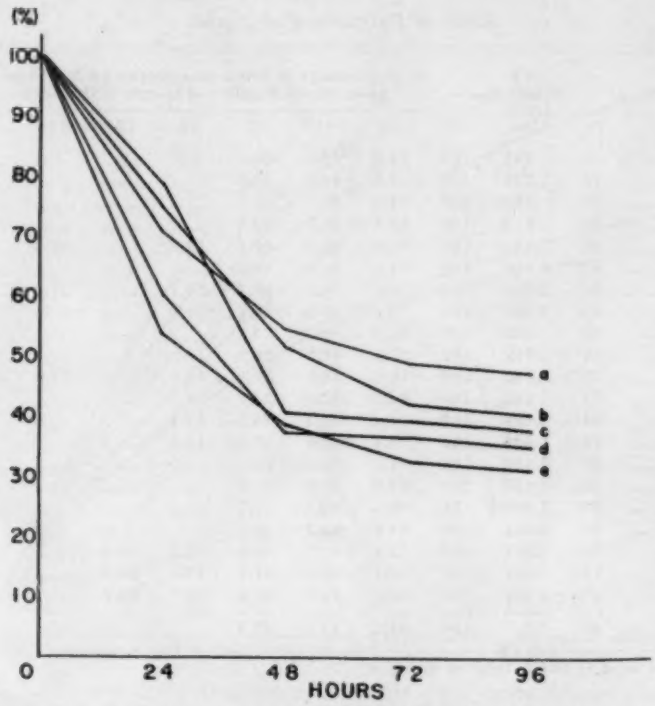

Fig. 4.—Curves showing the coordinate relationships between the losses of weight of 5 aortas and the periods of extraction with 89 per cent formic acid at 45 C. The weight of the residue of each aorta expressed in percentage of the initial weight of the intact specimen is plotted on the ordinate. The period of extraction is plotted in hours along the abscissa.

elastic tissue may be the only significant residue. In these instances, two of which are illustrated in figure 4, the loss of weight of the residue in the forty-eight to seventy-two hour interval seems to be attributable to solution of elastic tissue. When young aortas are dealt with, this is the usual finding. In studies of thick, fibrous, old aortas, a constant rate of loss of weight is not reached before the end of seventy-two hours' extraction. This is represented by three curves in figure 4.

Hence, in the series of 21 aortas the slopes of all curves in the seventy-two to ninety-six hour interval are essentially equal and remain constant in any extraction interval thereafter. Many weeks pass before the elastic tissue is wholly dissolved. The exact time at which absolute purity is reached is not known. A standard high degree of purity is approached at the end of seventy-two hours. For chemical purposes these residues must be subjected to further purification. The data which illustrate the various rates of purification of 21 aortas, aged 0.03 to 77 years, are recorded in the accompanying table.

Rates of Purification of Aortas

Number	Age, Yr.	Dry Weight of Aorta, Gm.	Percentage of Aorta Recovered as Residue After Given Number of Hours' Extraction							
			0	24	48	72	96	120	144	168*
24	.03	.181	100	36.1	31.8	28.9	.....	.....	.....	.....
38	12	1.227	100	47.5	44.2	40.6	.....	.....	.....	.....
55	28	.983	100	51.4	46.7	41.1	.....	.....	.....	.....
42	35	1.413	100	58.6	48.3	40.2	.....	.....	.....	.....
41	41	1.167	100	75.4	51.8	40.4	38.3	.....	33.9	.....
52	42	1.140	100	61.0	46.0	40.9	.....	.....	.....	.....
34	44	1.282	100	54.6	34.3	30.7	29.1	.....	27.3	.....
39	45	1.268	100	53.7	39.2	34.2	31.2	.....	.....	(e)
53	50	.983	100	78.2	40.7	38.2	.....	.....	.....	.....
47	51	1.019	100	72.4	61.2	40.2	.....	.....	.....	.....
26	53	1.442	100	60.1	38.6	37.4	35.0	33.1	31.0	29.1(d)
45	53	1.143	100	60.2	37.7	36.3	34.4	.....	.....	28.3
27	54	1.164	100	78.4	41.2	38.5	37.4	.....	.....	(c)
43	54	1.483	100	75.1	51.8	42.2	40.5	.....	.....	(b)
40	58	2.193	100	58.7	36.2	30.1	.....	.....	.....	.....
51	58	1.105	100	64.6	47.6	41.9	.....	.....	.....	.....
48	62	1.500	100	68.1	45.1	41.7	.....	.....	.....	.....
25	63	1.321	100	61.2	48.2	40.7	.....	.....	.....	.....
31	70	1.824	100	71.4	49.7	41.5	38.1	36.4	.....	.....
46	71	1.617	100	53.7	39.2	31.1	30.4	28.9	.....	.....
49	77	1.474	100	56.8	44.7	39.0	36.7	34.2	.....	.....
Average	49		100	61.8	44.0	37.9				

*Letters *e*, *d*, *c* and *b* refer to figure 4.

The loss of elastic tissue during extraction is reflected by changes in the forms of the elasticity curves. A series of curves of cylindric rings from 1 aorta is shown in figure 5. At a standard load the amplitude of the curve increases in rough direct proportion to the decrease in the amount of elastic tissue after reasonable purification. Nonelastic components, though they may be present in large quantities in the wall after twenty-four hours' extraction, have no appreciable effect on the forms of curves. Hence, in the average case the amplitude of the elasticity curve of segments after seventy-two hours' extraction can be predicted accurately if the amplitude after forty-eight hours' extraction is known. In individual cases, especially when old aortas are under investigation, the prediction is often subject to considerable error.

The decrease of tensile strength of purified networks in the average case is directly proportional to the duration of extraction. The slopes of curves which represent the rates of decrease in tensile strength are, however, about twice as great as the slopes of curves which represent the rate of solution of elastic tissue. From this it may be inferred that the degradation of elastic tissue as measured by its fragility is more rapid than the degradation as measured by solubility. A qualitative idea of the relationships between amplitudes of curves, fragility, maxi-

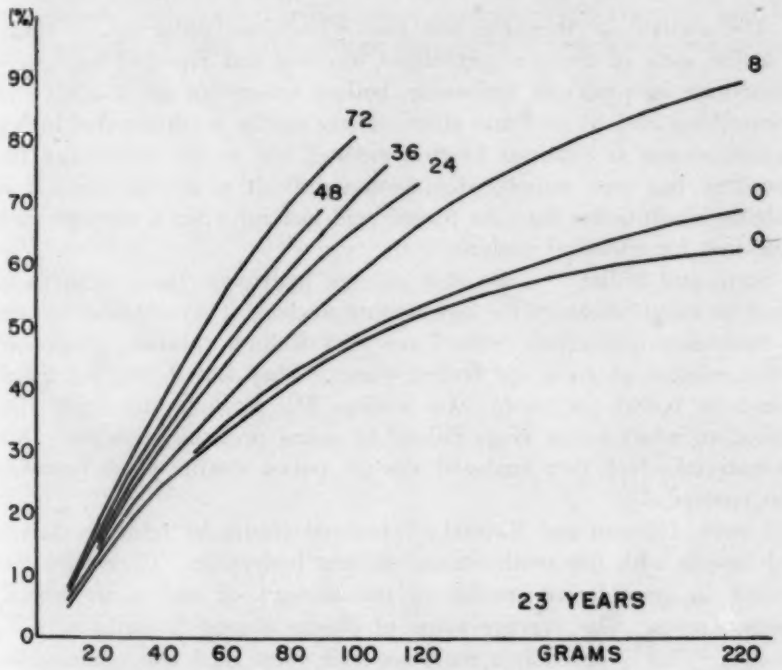

Fig. 5.—Curves illustrating the extensibility of a series of contiguous aortic rings after extraction with 89 per cent formic acid at 45 C. The extension of each ring in percentage of the original resting circumference is plotted on the ordinate. The load is plotted in grams along the abscissa. The number at the end of each curve refers to the time of extraction in hours. The two zero curves represent the range of initial measurements of all rings. These curves do not terminate within the limits of the loads placed on the rings. All other curves terminate at points of maximum tolerated loads and hence at the limits of maximum extension. The actual quality of the elastic tissue of this 23 year old aorta, so far as elongation in response to increments of load, maximum extensibility and fragility are measurements of quality, is represented by the seventy-two hour curve.

mum extensibility and rate of solution of elastic tissue may be gained by a comparison of the average figures in the table with the curves in figure 5.

The maximum extensibility of the isolated networks, as a rule, is well maintained at a constant figure, despite prolonged extraction.

The minimum retractility of isolated networks after removal of load likewise is not appreciably changed by prolongation of the period of extraction.

Data which will illustrate these general statements in a quantitative way will be presented in a subsequent report.

COMPARISON OF THE PRESENT METHOD WITH OTHER METHODS FOR THE ISOLATION OF ELASTIC TISSUE

The method of Richards and Gies [2a] involves reduction of tissue to a fine state of division, prolonged washing and repeated successive extractions with calcium hydroxide, boiling water, hot acetic acid, cold hydrochloric acid, alcohol and ether. There can be no doubt that highly purified elastin is obtained by this method, but in my experience the procedure has been time-consuming and difficult to standardize. It is probably much better than the formic acid method when a pure product is desired for chemical analysis.

Stein and Miller [2b] stated that calcium hydroxide has a deleterious action on elastic tissue of the ligamentum nuchae. They obtained elastin by successive extractions with 5 per cent sodium chloride, phosphate buffer solution at p_H 8 and boiling water. They stated that the tissue should be boiled for about two weeks. My attempts to apply this method to intact aortic rings failed. It seems probable, however, that the material which they analyzed was the purest elastin which has ever been isolated.

Lowry, Gilligan and Katersky [2c] isolated elastin by treating macerated tissues with hot tenth-normal sodium hydroxide. They used the method in quantitative studies of the amount of elastin in several human tissues. The average value of elastin among 5 aortas was 30 per cent. This is probably a more accurate value than that obtained by the method which I have described. However, if chemical analyses are planned, it is advisable not to use strong alkalis in the purification of elastin.[3] Furthermore, I may add from personal experience that strong alkalis should not be used, if physical measurements of the behavior of the isolated product under tension are contemplated.

SUMMARY

If fresh or dehydrated human aortas are extracted with 89 per cent formic acid for seventy-two hours at 45 C., ninety to ninety-five per cent of the elastic tissue is recovered as a purified residue.

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3. Richards and Gies.[2a] Stein and Miller.[2b]

The elastic networks isolated by extraction of the nonelastic components retain normal staining reactions and microscopic continuity of fibrils.

The isolated lamellas of the media often have angulate or spiral arrangements of discrete networks that are invisible in preparations of the intact aorta.

During extraction with formic acid at zero load there is spontaneous elongation of networks to an extent which approaches the elongation in response to the application of high mechanical tension. On neutralization of the purified acidic networks they retract to near normal dimensions and display properties of extensibility, retractility and tensile strength of such high orders that quantitative measurements may easily be made.

HEART WEIGHT

I. THE WEIGHT OF THE NORMAL HUMAN HEART

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CINCINNATI

The purpose of this investigation is to find a practical method for calculating what a normal heart in a given person should weigh and within what range that expected weight may vary without the alteration being evidence of atrophy or of hypertrophy. The standards for normal cardiac weight given in textbooks of anatomy and pathology and in periodical literature differ so widely that by their use one cannot recognize the lesser degrees of myocardial hypertrophy and atrophy (table 1).

Since the early stages of these processes are so obscure both clinically and pathologically, there exists much difference of opinion concerning the etiologic role played by certain conditions, among which are coronary sclerosis, pericardial adhesions, glandular dyscrasias and vitamin deficiencies. It is hoped that more accurate and more generally utilized criteria for slight departures from the normal heart weight may provide data for diagnosing myocardial hypertrophy in its early stages, may establish a basis for the study of cardiac atrophy and may lead to a better understanding of the causes of these two conditions. Detailed reviews of the literature concerning normal heart weight have been recorded by Smith,[1] Bedford and Treadgold,[2] Stieve,[2a] Bardeen [3] and many others.

Several methods have been used in the past to determine standards for heart weight: (1) Calculation of the average weight of hearts from unselected adults dying from any cause (Shennan [4]). The sources of error in this method are obvious. (2) Calculation of the average heart weight in series of cases selected because presumably the hearts were

From the Department of Pathology of the University of Cincinnati and the Cincinnati General Hospital.

1. Smith, H. L.: Am. Heart J. **4**:79, 1928.

2. Bedford, D. E., and Treadgold, H. A.: Lancet **2**:836, 1931. Further tests of the conclusions of this report were published in 1932 by Treadgold and H. L. Burton (Lancet **1**:277, 1932).

2a. Stieve, H.: Med. Klin. **34**:5 and 42, 1938.

3. Bardeen, C. R.: Am. J. Anat. **23**:423, 1918.

4. Shennan, T.: Post Mortems and Morbid Anatomy, ed. 3, Baltimore, William Wood & Company, 1935.

normal. The reported criteria for the "normal heart" have been concerned mainly with ruling out the obviously hypertrophied ones, while little attention has been given to the ones which may have been smaller than normal. Also, few, if any, reports present convincing evidence that all cases of hypertension have been excluded from the series of cases used. (3) Calculation of the heart weight/body weight ratio. It has long been known that the size of the body has some effect on the size of the heart. Since weight has been accepted generally as the most satisfactory measurement of heart size in regard to criteria for myocardial hypertrophy, it has been considered statistically proper to correlate heart weight with body weight. This correlation has been made by many investigators. The results have not been uniform because the many factors which may exert an effect on heart weight were not constant in all the series of cases used.

The effect of sex on heart weight has been recognized by most investigators in recent years. Other factors being constant, female hearts have usually been found to be smaller than male hearts (table 1).

There is more difference of opinion concerning the effect of age on heart weight. Gray [5] wrote: "The heart continues to increase in weight and size up to an advanced period in life, and this increase is more marked in men than in women." Piersol [6] stated: "The weight of the heart increases with age up to about the seventieth year, probably a slight diminution taking place after that period." Smith [1] stated that the weight of the heart does not increase with age except as the weight of the body increases. Aschoff [7] found no correlation between age and heart weight in his series of 468 soldiers. Comeau and White [8] stated that in the adult age was of no value as a correlation factor in studying heart size as determined by orthodiascopic measurements. Greenwood [9] studied 358 apparently normal hearts and concluded that the age increase amounted to about 9.4 Gm. for each decade. Bell and Hartzell [10] studied the effect of age on the size of the heart, but they included cases of hypertension, coronary sclerosis and other conditions affecting the heart, so that the data presented by their cases formed too complicated a picture to give convincing support to their statement that "there is therefore no satisfactory evidence of a correlation of heart weight with age alone."

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5. Gray, H.: Anatomy, Descriptive and Applied, edited by T. B. Johnson, ed. 26, New York, Longmans, Green & Co., 1935.

6. Piersol, G. A.: Human Anatomy, ed. 11, Philadelphia, J. B. Lippincott Company, 1916.

7. Aschoff, L.: Lectures in Pathology, New York, Paul B. Hoeber, 1924.

8. Comeau, W. J., and White, P. D.: Am. Heart J. **17**:616, 1939.

9. Greenwood, M., and Brown, J.: Biometrika **9**:473, 1913.

10. Bell, E. T., and Hartzell, T. B.: J. M. Research **44**:473, 1924.

TABLE 1.—*Previously Reported Heart Weight Standards*

Author	Heart Weight: Average, Gm. Males	Average, Gm. Females	Range, Gm. Males	Range, Gm. Females	Percentage of Body Weight: Males	Females	Unspecified
Arnold *	290	260	250-325	225-300	.....	.....	.....
Bardeen [3]	.....	.....	.....	.....	0.55	0.53	.....
Boyd *	.....	.....	.....	.....	0.59-0.84	0.51-0.80	.....
Cunningham *	310	255	.....	.....	.....	.....	0.488
Gray *	.....	.....	280-340	230-280	.....	.....	.....
Greenwood and Brown [9]	.....	.....	.....	.....	.....	.....	0.575 (0.45-0.70)
Jackson *	312	255	.....	.....	0.55	0.53	.....
Kaufmann *	300	250	.....	.....	.....	.....	.....
Müller *	.....	.....	.....	.....	0.55-0.75	0.50-0.69	.....
Peacock *	.....	.....	227-312	198-284	.....	.....	.....
Piersol [6]	312	274	266-346	230-340	0.59	0.61	.....
Saphir *	300	250	.....	.....	.....	.....	.....
Shennan *	384	326	.....	.....	0.60	0.63	.....
Smith *	294	250	155-400	110-367	0.43	0.40	.....
Thoma *	.....	.....	.....	.....	.....	.....	0.463
Vierordt *	.....	.....	275-367	264-310	.....	.....	.....
White *	300	250	.....	.....	0.40	0.45	..... (0.35-0.50)

* Reference and comment are given below.

Arnold, H. D.: M. & S. Rep. Boston City Hosp., 1899, ser. 10, p. 83: "Weights for 25 grams outside these limits may be normal; beyond that they are almost surely abnormal."

Boyd, cited by Bell and Hartzell.[10]

Cunningham, D. J.: Manual of Practical Anatomy, ed 2, New York, William Wood & Co., 1923: "The weight varies greatly, always, however, in definite relation to the weight of the body, the relative proportion changing at different periods of life."

Gray [5]: "It continues to increase in weight and size up to an advanced period in life, and this increase is more marked in men than in women."

Jackson, C. M.: The Effects of Inanition and Malnutrition upon Growth and Structure, Philadelphia, P. Blakiston's Son & Co., 1925. Morris' Human Anatomy, ed. 8, Philadelphia, P. Blakiston's Son & Co., 1925. This applies only to cases "of average build." "In emaciated individuals it usually weighs relatively more, and relatively less in the fat."

Kaufmann, E.: Pathology, Philadelphia, P. Blakiston's Son & Co., 1929.

Müller, W.: Die Massenverhältnisse des menschlichen Herzens, Leipzig, Leopold Voss, 1883.

Peacock, T. B.: Monthly J. M. Sc. **19**: 193, 313 and 403, 1854.

Saphir, O.: Autopsy Diagnosis and Technique, New York, Paul B. Hoeber, Inc., 1937.

Shennan [4]: Calculated from 1,020 cases of patients dying from all causes. Hearts varied in weight from 184.25 to 1,445 Gm.

Smith [1]: The ratio is slightly higher in thin persons and lower in the obese.

Thoma, R.: Untersuchungen über die Grösse und das Gewicht der anatomischen Bestandtheile des menschlichen Körpers im gesunden und kranken Zustände, Leipzig. F. C. W. Vogel, 1882.

Vierordt, K. H.: Anatomische, physiologische und physikalische Daten und Tabellen, ed. 3, Jena, Gustav Fischer, 1906.

White, P. D.: Heart Disease, ed. 2, New York, The Macmillan Company, 1937: "Enlargement up to 425 grams may exist in a large individual without clinical signs, but hypertrophy of the heart beyond that weight should be found by clinical study."

Some of the variations in the results obtained by different investigators using the heart weight/body weight ratio may be explained by the types and number of cases included in their series. Many of the

reported series of cases were so small and the variation in heart weight so great that, had the probable error of the mean been calculated, no significance could have been attached to the mean heart weight. Among the investigators using larger series of cases was H. L. Smith,[1] who studied 1,000 cases presenting none of the well recognized causes of myocardial hypertrophy. He concluded that the weight of the normal heart may be calculated from the weight of the body with an error of about 8 to 10 per cent. The ratio was higher with thin persons and lower with obese ones. He stated that this method is not so accurate for the body weight under 100 pounds (45.5 Kg.) or over 210 pounds (95 Kg.). From the results of Smith and others it seems that the heart weight/body weight ratio would be a satisfactory criterion for normal heart weight only when calculated from data on a large series of persons who are of relatively normal body weight and who present no clinical or pathologic evidence of any of the known causes of myocardial hypertrophy or atrophy and no clinical or pathologic evidence of heart disease. In addition, separate ratios should be calculated for each sex; also, age and color should be investigated as possible contributing factors. No report has been found in the literature which presents mean heart weight/body weight ratios of statistically proved reliability, with all of these factors evaluated. Aschoff's group of soldiers consisted of adults of the same sex and of similar body build and body nourishment, dying of causes not likely to affect heart weight; so his series provides a standard which should be quite accurate for males of similar body build and nourishment. In Smith's series of 1,000 cases not many were emaciated or obese. Among 532 adult males he reported 24 weighing over 190 pounds (86 Kg.) and 33 weighing less than 120 pounds (54.5 Kg.).

The method of calculating heart weight as a percentage of the body weight has certain disadvantages. Autopsies are frequently performed in laboratories, funeral parlors or other places where there are no facilities for weighing cadavers. It is much easier to provide facilities for weighing viscera than for weighing the entire body. Moreover, body weight is not stable. Variation in body weight is an integral part of the picture presented by many disease processes. It would be desirable to use only normal body weights in calculating standards for normal heart weight. But what is normal body weight? Whatever it is, it is frequently not presented at autopsy, or even clinically during the last illness, except in healthy persons dying suddenly of accidental causes. Such factors as terminal dehydration, ascites, edema, urinary retention, large "fibroids," ovarian cysts, pregnancy, loss of a limb and other causes of increase or decrease in body weight may not cause a proportional change in heart weight. In fact, many of these conditions probably do not affect heart weight at all. It would be more accurate to use the weight of the body during health, but that figure is usually not available

at autopsy, and therefore cannot be used as a basis for judging whether or not there is myocardial hypertrophy or atrophy.

Some investigators have estimated the normal weight for a given body from the height, sex and age, using insurance tables as their standard, and have used this estimated body weight in calculating the normal heart weight/body weight ratio. However, these reported series of cases included emaciated and obese persons, as well as normally nourished ones. If emaciation and obesity affect heart weight, the hearts of these persons were not suitable material for calculating standards for normal heart weight, even though a corrected body weight was used in the ratio. The quantitative effect of emaciation and of obesity on the heart weight/body weight ratio has not been determined. The reports on this subject present conflicting points of view. Results from experiments on small series of experimental animals range from no loss in heart weight with loss in body weight to a percentage loss in heart weight greater than the percentage loss in body weight. Van Liere and Sleeth,[11] after working with 33 guinea pigs, concluded: "In prolonged inanition, the heart loses slightly more weight than does the body." The percentage loss in their animals was so slight as to be within the range of experimental error. In man the heart weight/body weight ratio seems to increase slightly with emaciation and decrease with obesity (table 1: Jackson; Smith). Since obesity and emaciation are parts of various disease conditions it seems advisable to consider tentatively, at least, that variations in heart weight associated with these changes in the state of body nourishment are departures from normal in heart weight.

Many attempts have been made to diagnose degrees of myocardial hypertrophy by methods not requiring the use of heart weight, the most common method being clinical determination of heart size by roentgen examination or by percussion. Although much knowledge concerning variation in heart size has been acquired by these methods, it must be remembered that "size" thus measured means dimensions and is not synonymous or even directly correlated with heart weight. A dilated heart is a large heart, but its weight may not be increased in proportion to its increase in dimensions. Increase in dimensions is not an accurate criterion of myocardial hypertrophy, which is an increase in the size of the heart muscle cells and thus an increase in muscle bulk. Increase in cardiac dimensions may be an increase only in the size of the heart's cavities with little or no increase in muscle bulk or in heart weight. Other factors which may cause variations in heart weight, such as fluid, fibrous tissue and inflammatory exudates, probably produce changes in weight too small to be differentiated at present from variations in normal heart weight. At present there is no clinical method by which heart weight can be accurately determined or predicted.

11. Van Liere, E. J., and Sleeth, C. K.: Am. J. Physiol. **116**:635, 1936.

In summarizing methods of calculating heart weight standards and in diagnosing departures from normal in heart weight, the best method in present use seems to be the heart weight/body weight ratio method but, as has been pointed out, this method is often inaccurate, impracticable and sometimes impossible to use.

Height, or body length, as measured at autopsy, occasionally has been considered a possible factor in estimating heart weight. In a group of healthy persons considerable differences in body weight can be expected to be associated with differences in height. Variations in body weight to the degrees commonly designated as emaciation and obesity are not accompanied by comparable changes in height. In other words, variation in body weight not due to pathologic conditions is directly correlated with variation in height when the factors age and sex remain constant (Dorst,[12] citing tables of the Medico-Actuarial Mortality Investigation). Therefore, it seems reasonable that any variation in heart weight with body weight which is not part of a pathologic condition would be also a variation with height.

Height is a much more stable factor in body "build" than is weight and is much less influenced by disease processes. At autopsy it is much easier to obtain an accurate measurement of body length than of body weight.

A search through the available literature revealed many suggestions of possible correlation between height and heart weight. In 1878 Beneke[13] studied the "volume of the heart substance from the standpoint of body length." Smith included in his report a graph showing the close correlation between heart weight and height—but he dismissed it with the remark that "the height affects the heart weight only as the height effects the body weight." In 1930 Kirsch[14a] traced through youth the parallelism between increase in the breadth and the length of the heart and increase in body height. In 1931 Bedford and Treadgold[2] used Eyster's[15] formula to predict the normal transverse diameter of the heart as seen in roentgenograms of the living subject. This formula includes factors for both height and weight of the body. He concluded that TD (transverse diameter) can be predicted from body weight alone in those of average height for weight, but that in others both height and weight must be taken into account to obtain an equally successful prediction.

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12. Dorst, S. E., in Piersol, G. M.; Bortz, E. L., and others: Cyclopedia of Medicine, Philadelphia, F. A. Davis Company, 1931, vol. 2, p. 534.

13. Beneke, F. W.: Die anatomischen Grundlagen der Constitutionsanomalieen des Menschen, Marburg, N. G. Elwert, 1878.

14. Kirsch, O.: (*a*) Klin. Wchnschr. **9**:881, 1930; (*b*) Jahrb. f. Kinderh. **137**: 185, 1932.

15. Eyster, J. A. E.: Radiology **8**:300, 1927; Arch. Int. Med. **41**:667, 1928; Tr. A. Am. Physicians **43**:18, 1928

In 1932 Kirsch [14b] again mentioned body length as one of the factors to be considered in evaluating the size of the heart. During that year Moritz [16] also correlated body length with heart size, but he used, not the weight, but the breadth and the length of the heart as measurements of size. In 1934 Bray [17] included height in a list of external dimensions which he sought to correlate with heart weight, but unfortunately must have included some diseased hearts, since they varied in weight from 102 to 480 Gm. In 1939 Comeau and White [8] studied the body build and heart size of twins. They stated that body weight and surface area were more important than height in determining heart size as seen by roentgenograms. In 1940 Fray [18] described his cardiomensurator, an instrument for the detection of cardiac enlargement in roentgen films, and he made use of "the direct correlation of transverse diameter of the heart with body weight and height."

PRESENT INVESTIGATION

In order to determine the possible relationship between height and heart weight, and also to learn more about factors related to heart weight, the present study was begun. From the autopsy records of the Cincinnati General Hospital a series of 933 cases was collected in which the hearts had been considered relatively normal both clinically and pathologically and in which none of the well recognized causes of myocardial hypertrophy were present.

The criteria for the inclusion of cases in this series were as follows:

(1) No gross or microscopic lesions in the heart or the pericardium except
  (*a*) toxic changes, such as edema or cloudy swelling, and
  (*b*) relatively unimportant minor departures from normal, such as "slight fibrosis," "milk patch in the epicardium" or "slight atheroma of valves."

(2) No clinical evidence of cardiac disease except terminal acute cardiac dilatation.

(3) No gross or microscopic evidence of lesions causing increased peripheral resistance in any considerable extent of the peripheral vascular bed, such as extensive fibrosis of an organ or constricting lesions in arterioles.

(4) No recorded systolic blood pressure exceeding 140 or diastolic pressure exceeding 90.

(5) No deforming lesions which may have affected height, such as scoliosis.

(6) All persons 21 years of age or older.

An examination of the records of 9,676 consecutive cases in which autopsy was done at the Cincinnati General Hospital from 1924 to 1940, inclusive, revealed only 933 cases which fulfilled the foregoing criteria.

16. Moritz, F.: Deutsches Arch. f. klin. Med. **174**:330, 1932.
17. Bray, E.: Arch. ital. di anat. e di embriol. **32**:257, 1934.
18. Fray, W. W.: Am. Heart J. **19**:417, 1940.

In order to rule out arteriolosclerosis as having a possible effect on the heart weight, microscopic sections of heart, pancreas, kidney, liver, spleen and adrenal were searched for this lesion in each case. Cases which fulfilled all of the stated criteria except for the presence of arteriolosclerosis (and which therefore were excluded from this series) will be dealt with in a subsequent report.

In the pathologic laboratory of the Cincinnati General Hospital, where in each of these cases autopsy was done, it is customary to measure the body length along the flat surface on which the body is supine. The heart is weighed after its great vessels have been severed near their pericardial attachments and after the heart has been opened and the clots removed. The body weights are not recorded.

The factors chosen for investigation as possibly having an effect on heart weight were sex, color, age and state of body nourishment. In all cases used, both the autopsy and the clinical records contained descriptions of the state of nourishment. After dividing the cases into two groups according to sex, we redivided them according to race. Three males and four females were designated mulattoes in the records; so their cases were dropped from this series, leaving 926 cases for further study. It is understood that in this series there probably were others who were not as pure white or Negro as the records would indicate. This probability must be remembered when evaluating the racial factor in this report.

The race-sex groups were then subdivided as follows: (1) cases in which the body was described as "emaciated," "wasted" or "cachectic;" (2) cases in which the body was described as "obese"; (3) cases in which the body was described as "powerfully developed muscularly"; (4) cases not included in the above categories, classed as cases in which the body was normally nourished.

It is fully realized that group 4 may contain some cases belonging in one of the other groups, but since most of the cases were described by two or more independent observers, errors of this type are probably not numerous.

Table 2 indicates that in this series of 926 cases no significant difference in heart weight was found between white persons and Negroes. The female hearts were significantly lighter in weight than the male hearts in each category. Hearts from emaciated persons were significantly lighter in weight than hearts from better nourished persons of the same sex. Hearts from obese persons and from those of unusually powerful muscular development were heavier than those from persons in a more nearly average state of nutrition and muscular development. Although only 12 persons of unusually powerful muscular development were studied, all males, 8 of them had hearts weighing 375 Gm. or more, the largest being 425 Gm.

Therefore, sex, state of nourishment and probably muscular development of unusual degree must be considered in calculating standards for normal heart weight.

TABLE 2.—*Effect of Sex, Race and State of Body Nourishment on Heart Weight*

		Males					Females				
Race	Physical Classification	Number	Mean Heart Weight, Gm.	Probable Error of Mean, Gm.	Standard Deviation, Gm.	Coefficient of Variation, per Cent	Number	Mean Heart Weight, Gm.	Probable Error of Mean, Gm.	Standard Deviation, Gm.	Coefficient of Variation, per Cent
Negroes	Emaciated	71	267.5	3.9	49.0	18.3	63	220.4	3.1	36.0	16.3
	Normally nourished	141	326.4	2.3	40.8	12.5	94	263.7	2.8	41.0	15.5
	Obese	8	357.5	9.2	38.2	10.7	25	289.2	3.3	29.2	10.1
	Muscular	5	367.0	12.1	39.3	10.7	.....	.....	.....	.....	.....
	Total	225	310.5	2.4	52.8	17.0	182	252.2	2.4	47.4	18.8
White persons	Emaciated	65	261.0	3.7	44.3	17.0	58	216.0	3.1	35.2	16.3
	Normally nourished	216	325.8	1.9	41.1	12.6	130	261.1	2.2	37.1	14.2
	Obese	10	354.0	10.0	46.0	13.0	33	289.7	4.9	34.9	12.1
	Muscular	7	379.3	5.2	19.5	5.3	.....	.....	.....	.....	.....
	Total	298	313.5	2.0	51.8	16.5	221	253.5	1.9	41.4	16.3
Total	Emaciated	136	264.9	2.7	47.0	17.7	121	218.3	2.4	38.4	17.6
	Normally nourished	357	319.3	1.3	40.9	12.8	224	262.2	1.4	30.2	11.5
	Obese	18	355.6	6.8	42.8	12.0	58	289.5	2.9	32.6	11.3
	Muscular	12	374.2	5.8	30.0	8.0	.....	.....	.....	.....	.....
	Total	523	312.0	1.7	52.0	16.6	403	252.9	1.6	46.4	18.3

TABLE 3.—*Age and Heart Weight in Normally Nourished Cadavers*

	Males			Females		
Age, Yr.	Number	Mean Heart Weight, Gm.	Probable Error of Mean, Gm.	Number	Mean Heart Weight, Gm.	Probable Error of Mean, Gm.
21-29	99	315.8	2.7	100	254.9	2.7
30-39	111	322.3	2.6	78	263.3	3.3
40-49	90	318.3	2.8	36	276.0	4.6
50-59	39	319.5	4.3	6 *	295.0	.....
60-69	15	318.7	6.9	4 *	248.8	.....
70-79	3 *	350.0	.....	.....	.....	.....

* Here there are too few cases to be useful. Pearl [19] stated that when a mean is based on less than fifteen observations, special methods must be used to determine its reliability.

In order to determine whether age has any direct influence on heart weight, table 3 was constructed.

Using the simple theorem in probability which states that the probable error of the difference between two independent quantities is equal to the square root of the sum of the squares of the probable errors of the quantities entering into the differences (Pearl [19]), and realizing

19. Pearl, R.: Introduction to Medical Biometry and Statistics, ed. 2, Philadelphia, W. B. Saunders Company, 1930.

also that a difference between two quantities, to be significant, must be four or more times its probable error, I used the following formula to test the significances of the differences between the various mean heart weights in table 3.

$$\frac{\text{Mean}_A - \text{Mean}_B}{(\text{P.E.}_A)^2 + (\text{P.E.}_B)^2}$$

TABLE 4.—*Heart Weight and Body Length in Normally Nourished Males*

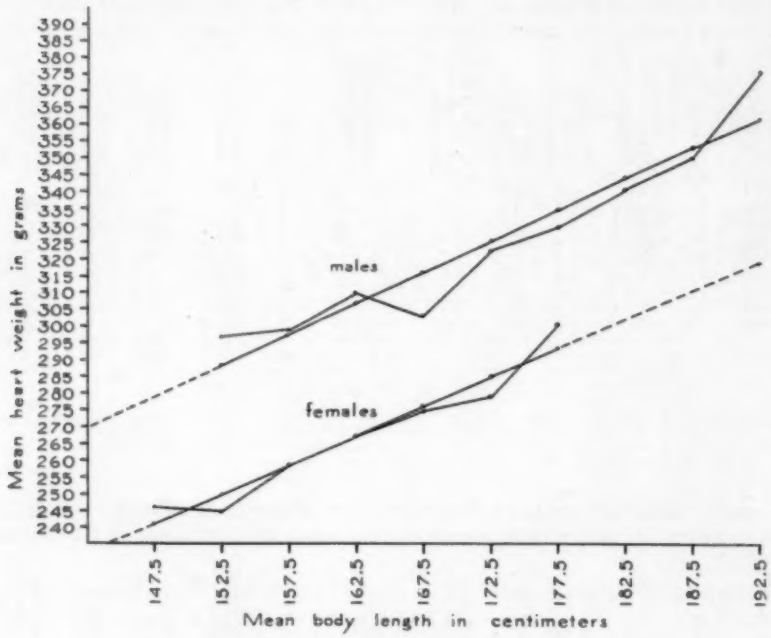
Heart Weight, Gm.	Cases	Mean Body Length, Cm.	Probable Error of Mean, Cm.	Standard Deviation, Cm.	Coefficient of Variation, per Cent	Body Length, Cm.	Cases	Mean Heart Weight, Gm.	Probable Error of Mean, Gm.	Standard Deviation, Gm.	Coefficient of Variation, per Cent
200-224	3	165.7	1.3	3.3	2.0	140-144	1	250	.....	.....	.....
225-249	11	168.0	0.9	4.5	2.7	145-149	0	.....	.....	.....	.....
250-274	29	168.2	1.2	9.7	5.8	150-154	6	296.7	10.0	36.5	13.7
275-299	49	167.3	0.6	6.7	4.0	155-159	11	298.1	4.7	23.3	7.8
300-324	89	170.6	0.5	7.2	4.2	160-164	51	309.8	3.0	32.0	10.3
325-349	64	170.4	0.6	6.7	3.9	165-169	84	303.2	2.5	37.7	12.4
350-374	76	171.7	0.4	5.7	3.3	170-174	99	322.8	2.4	34.8	10.8
375-399	23	174.1	0.9	6.4	3.7	175-179	62	330.4	2.5	40.9	12.4
400-424	13	186.0	1.3	6.7	3.6	180-184	31	341.8	4.9	40.7	11.9
						185-189	8	351.3	12.0	49.8	14.2
						190-194	3	376.7	15.7	40.3	10.7
						195-199	0	.....	.....	.....	.....
						200-204	1	240.0	.....	.....	.....
Total	357						357	319.3	1.5	40.9	12.8

TABLE 5.—*Heart Weight and Body Length in Normally Nourished Females*

Heart Weight, Gm.	Cases	Mean Body Length, Cm.	Probable Error of Mean, Cm.	Standard Deviation, Cm.	Coefficient of Variation, per Cent	Body Length, Cm.	Cases	Mean Heart Weight, Gm.	Probable Error of Mean, Gm.	Standard Deviation, Gm.	Coefficient of Variation, per Cent
150-174	3	153.7	0.5	1.3	0.8	135-139	1	250.0	.....	.....	.....
175-199	6	152.2	1.0	3.5	2.3	140-144	1	240.0	.....	.....	.....
200-224	25	157.7	0.9	7.0	4.4	145-149	15	246.3	6.6	38.1	15.5
225-249	42	157.4	0.7	7.2	4.6	150-154	43	244.9	4.3	41.6	17.0
250-274	55	159.6	0.7	7.4	4.6	155-159	53	259.3	3.6	38.7	14.9
275-299	41	160.9	0.7	6.9	4.3	160-164	61	267.0	3.5	41.3	15.5
300-324	30	161.3	0.8	6.3	3.9	165-169	29	274.5	4.6	36.4	13.3
325-349	15	161.5	1.4	8.2	5.1	170-174	13	278.5	8.1	43.2	15.5
350-374	7	166.6	1.5	5.8	3.5	175-179	8	300.6	8.6	35.6	11.8
Totals	224						224	262.2	1.4	30.2	11.3

Since within each sex group the difference between the largest and the smallest mean heart weight (of age groups including 15 or more cases) was less than four times its probable error, the conclusion is that changes in age between 21 and 69 years in males and between 21 and 49 years in females have no direct effect on heart weight. More cases in the older age groups must be studied before further conclusions are justifiable.

Since age and race were not found to exert any influence on heart weight, all of the males and females were grouped, respectively, in two frequency distribution tables according to body length and heart weight (tables 4 and 5). The mean, the probable error of the mean, the standard deviation and the coefficient of variation were calculated for each heart weight class and for each body length class.



Heart weight and body length.

In the males, as heart weight increased from 200 to 424 Gm. there was an increase in mean body length from 165.7 to 186 cm. As body length increased from 150 to 194 cm., there was an increase in mean heart weight from 296.7 to 376.7 Gm. The coefficient of correlation between heart weight and body length in these 357 normally nourished males was 0.33 ± 0.03. This figure indicates definite but imperfect correlation.

In the female group, as the heart weight increased from 150 to 374 Gm., there was an increase in mean body length from 153.7 to 166.6 cm. As the body length increased from 145 to 179 cm., there was an increase in mean heart weight from 246.3 to 300.6 Gm. The coefficient of correlation between the heart weight and the body length in these 224 normally nourished females was 0.29 ± 0.04. This figure also indicates definite but imperfect correlation.

In order to check further the possible effect of race on heart weight, the data were rearranged in such a way as to keep the body length and sex factors constant. No significant difference in mean heart weight between white persons and Negroes was found in any of the body length groups. For example, 29 white males measuring 160 to 164 cm. had a mean heart weight of 309 Gm., while 22 Negro males of that body length group had a mean heart weight of 310 Gm. Among females with body lengths of 150 to 154 cm. the mean heart weight of 35 white females was 242, and that of 23 Negro females was 247 Gm. A similar rearrangement of data for the effect of age on heart weight, the body length and sex factors being kept constant, showed no significant difference in heart weight between the various decade age groups.

In the figure the mean heart weights for males and females are plotted against the variations in body length. In order to discount the fluctuations due to accidents of random sampling, the data were subjected to the mathematical process called curve fitting, the method of least squares being used. The straight line for each sex thus evolved has also been plotted in the figure. Any point on this line represents the most probable normal heart weight for a body of the length indicated at that point. However, it must be remembered that there can be deviations within the normal from any point on this line equal to the standard deviations given in tables 4 and 5. For example, a normal heart from a normally nourished female body measuring 150 cm. in length will most probably weigh 245 Gm. but may weigh any amount between 215 and 275 Gm. (245 ± 30) and still be considered of normal weight.

The equation to a straight line, $y = a + bx$, was used, letting $x =$ the middle point of each of the body length groups in tables 4 and 5, and y, the corresponding mean heart weights. Then, with the normal equations for fitting a straight line, which have been worked out by the principles of differential calculus, the equation $y = a + bx$ was solved for the values of a and b. Thus the following formulas were derived:

(1) For normally nourished adult males, the heart weight in grams equals 1.9 times the body length in centimeters minus 2.1 plus or minus 40.

$$\text{H.W.} = 1.9\ \text{B.L.} - 2.1 \pm 40$$

(2) For normally nourished adult females,

$$\text{H.W.} = 1.78\ \text{B.L.} - 21.58 \pm 30$$

With these formulas the heart weights for bodies of various lengths were calculated (table 6). This table provides a standard for judging whether or not a given heart in a given body is within normal weight limits. Furthermore, groups of cases with the same body length can be compared in order to determine whether or not there is any significant difference in heart weight between the groups. It is well to remember that to be significant a difference must be four or more times its probable error.

SUMMARY

Statistical analysis of the weights of hearts from 926 adult bodies in which was found no clinical or pathologic evidence of heart disease or of any commonly recognized cause of myocardial hypertrophy revealed the following factors to have an effect on heart weight; sex, body length and state of body nourishment. No effect of age or race on heart weight was demonstrated.

TABLE 6.—*Normal Heart Weights*

Body Length, Cm.	Heart Weight, Gm.		Body Length Cm.	Heart Weight, Gm.	
	Males $\sigma = \pm 40$	Females $\sigma = \pm 30$		Males $\sigma = \pm 40$	Females $\sigma = \pm 30$
135	254	219	168	317	277
136	256	220	169	319	279
137	258	222	170	321	281
138	260	224	171	323	283
139	262	226	172	325	284
140	264	227	173	327	286
141	266	229	174	329	288
142	268	231	175	330	290
143	270	233	176	332	291
144	272	235	177	334	293
145	273	236	178	336	295
146	275	238	179	338	297
147	277	240	180	340	299
148	279	242	181	342	300
149	281	243	182	344	302
150	283	245	183	346	304
151	285	247	184	348	306
152	287	249	185	349	307
153	289	251	186	351	309
154	291	252	187	353	311
155	292	254	188	355	313
156	294	256	189	357	315
157	296	258	190	359	316
158	298	259	191	361	318
159	300	261	192	363	320
160	302	263	193	365	322
161	304	265	194	367	323
162	306	267	195	368	325
163	308	268	196	370	327
164	310	270	197	372	329
165	311	272	198	374	331
166	313	274	199	376	332
167	315	275	200	378	334

σ = standard deviation.

In relatively normally nourished males the weight in grams of a normal heart was found to be 1.9 B. L. — 2.1 ± 40, B. L. being the body length in centimeters. The normal heart weight in normally nourished females was found to be 1.78 B. L. — 21.58 ± 30. Since emaciation and obesity are parts of pathologic processes, the variations in heart weights found to be associated with these conditions were considered departures from normal. Therefore, bodies presenting these conditions were not included in the series used for the determination of standards for normal heart weight. These standards based on body length were found to be more accurate and more useful than the commonly employed standards related to body weight.

PAPILLARY CYSTADENOMA LYMPHOMATOSUM

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Of the primary tumors of the salivary glands, none is more interesting than the comparatively uncommon neoplasm masquerading under a variety of names in the literature, and discussed by Lang [1] as papillary cystadenoma lymphomatosum of the salivary glands, but generally known simply as cystadenoma lymphomatosum. This tumor was first described in the literature, together with a group of cases of congenital epithelial cysts and fistulas by Hildebrand,[2] in 1895. Albrecht and Arzt,[3] in 1910, isolated this special type of tumor from the general group and described it as a papillary cystadenoma lymphomatosum. Since then cases have been reported under various designations by Ribbert, Spitznagel, Stohr and Risak, Lubarsch, Glass, Whartin, Jaffé, Harris, Wendel, Kraissl and Stout, Hall, Wood, Carmichael, Davie and Stewart. In 1938, Freshman and Kurland [4] reviewed the literature, collecting 54 recorded cases and added 1 of their own. In the same year Swinton and Warren [5] reported 5 more cases and 2 additional cases were described by Joyce, Menne and Heller [6] in 1941, bringing the total of the reported cases to 62 at the time of writing.

GENESIS

Several explanations have been offered for the origin of this interesting tumor. They are briefly reviewed in the following paragraphs.

Orbital Inclusion Cysts.—During investigations on the development of orbital glands in some species of carnivora, Kraissl and Stout [7]

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1. Lang, F. J., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1929, vol. 5, pt. 2, pp. 128-131.
2. Hildebrand, O.: Arch. f. klin. Chir. **49**:183, 1895.
3. Albrecht, H., and Arzt, L.: Frankfurt. Ztschr. f. Path. **4**:47, 1910.
4. Freshman, F. W., and Kurland, S. K.: Am. J. Clin. Path. **8**:422, 1938.
5. Swinton, N. W., and Warren, S.: Surg., Gynec. & Obst. **67**:424, 1938.
6. Joyce, T. M.; Menne, F. R., and Heller, W. E.: Arch. Surg. **42**:338, 1941.
7. Kraissl, C. J., and Stout, A. P.: Arch. Surg. **24**:485, 1933.

observed that the adult parotid gland develops from an embryonal sulcus in the oral cavity. They found that earlier investigators had reported tubular structures lined by tall columnar epithelium in the region of the parotid glands but not connected with these glands. The tubules were felt to be capable, under certain conditions, of developing into cystic structures. With the growth of the embryo, the parotid gland comes to lie in close proximity to the tubular structure, which they named the orbital inclusion. The ultimate fate of this structure in man has never been determined, but it is within the realm of possibility that such a closed vestigial epithelium-lined duct under certain conditions might develop into a cystic growth.

Oncocytic Origin.—Jaffé [8] proposed the theory that the tumor arises from oncocytes, cells described in the salivary glands first by Schaeffer and also by Hamperl.[9] These cells develop from the secreting epithelium and from the lining cells of the ducts by increasing in size and by differentiation of the cytoplasm, which assumes a finely granular appearance. The granules have only slight affinity for sudan III; they stain best with acid fuchsin and congo red. The nuclei are situated near the lumen and are rich in chromatin. The cells are rarely seen before the fourth decade of life. Jaffé did not believe that the lymphatic stroma is an essential part of the tumor. On the other hand, he felt that the tumor arose in the lymph nodes which are present about the glands. The absence of lymphadenoid structure was explained by the assumption that the normal pattern may be altered by the tumor. He was further supported in his views by the observations of others who found islands of salivary gland tissue in cervical lymph nodes. As pointed out by Harris,[10] one of the main objections to this theory is that oncocytes are seldom seen in people under 30 years of age, although the tumor has been described in persons of younger age periods.

Heterotopic Salivary Gland Rests in Lymphoid Tissue.—Albrecht and Arzt advanced the hypothesis that these tumors arise from misplacements of salivary gland tissue in lymph nodes. Support has been given to this idea by the studies of Chievitz, Neisse, Lowenstein, Thaysen and others on the development of the salivary glands, in which the acini and ducts of the glands and the lymphadenoid tissue are found in intimate relationship. Nodules of lymphatic tissue form within the glands, and in these formed islands glandular structures may subsequently appear. In addition to this process, areas of glandular tissue may become

8. Jaffé, R. H.: Am. J. Cancer **16**:1415, 1932.
9. Hamperl, H.: Virchows Arch. f. path. Anat. **282**:724, 1931.
10. Harris, P. N.: Am. J. Path. **13**:81, 1937.

enclosed in true lymph nodes. Lubarsch pointed out that islands of parotid or submaxillary gland tissue are occasionally found in the lymph nodes about the parotid glands, at the angles of the mouth or in the neck.

Branchiogenic Origin.—Among the proponents of the theory that the tumor is of branchiogenic origin were Askanazy,[11] Sternberg [11] and others. They based their views on the embryonal type of the epithelium and on the lymphoid stroma, which is characteristic of branchiogenic cysts.

Aberrant Anlage of the Eustachian Tube.—Warthin [12] concluded that these growths arise from heterotopic rests of ectodermal cells of the respiratory or eustachian tube anlages. He based this view on the embryologic character of the epithelium, which resembles closely that found in the eustachian tube. This view was supported by Wendel.[13]

REPORT OF CASES

CASE 1.—A man aged 66 entered the hospital Aug. 2, 1940 (service of Dr. M. A. Rabinowitz), complaining of difficulty in breathing and weakness of ten days' duration. He was known to have hypertension but felt well until ten days previously, when he was seized with substernal discomfort, nausea, vomiting and general malaise. His blood pressure was 170 systolic and 100 diastolic; with rest in bed, it fell to 120 systolic and 70 diastolic. The respirations were labored, and the pulse was slow; the temperature was 100.8 F. The heart was slightly enlarged, and the sounds were faint and of poor quality. Resonance at the base of the right lung was impaired and breath sounds were diminished, and there were fine crackling rales. A few small cervical lymph nodes were palpable, and one large mass at the angle of the left jaw. This mass was removed for histologic study. The patient made an uneventful recovery from this operation. With rest his other symptoms improved, and he was discharged from the hospital on September 22.

The specimen was an oval firm nodular mass, which measured 2.6 by 1.8 by 1.5 cm. The external surface was pink-red and was roughened by gray-yellow fibrous tags. In the cut surfaces it was homogeneous gray-brown, mottled with tiny areas of yellow.

The sections showed many branched papillary stalks of delicate vascular connective tissue on which were mounted columnar cells in two rows, uniform in size and shape and resting on a thin basement membrane (fig. 1 *A* and *B*). The borders were distinct, and the cytoplasm was abundant and pink; the nuclei were round or ovoid, deeply stained and situated near the luminal poles. In places they formed spaces containing amorphous pink and lavender staining material. The stroma was composed of a diffuse dense concentration of small

11. Askanazy, M., quoted by Sternberg, C., in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1929, vol. 1, pt. 1, pp. 335-336.

12. Warthin, A. S.: J. Cancer Research **13**:116, 1938.

13. Wendel, A.: J. Cancer Research **14**:123, 1930.

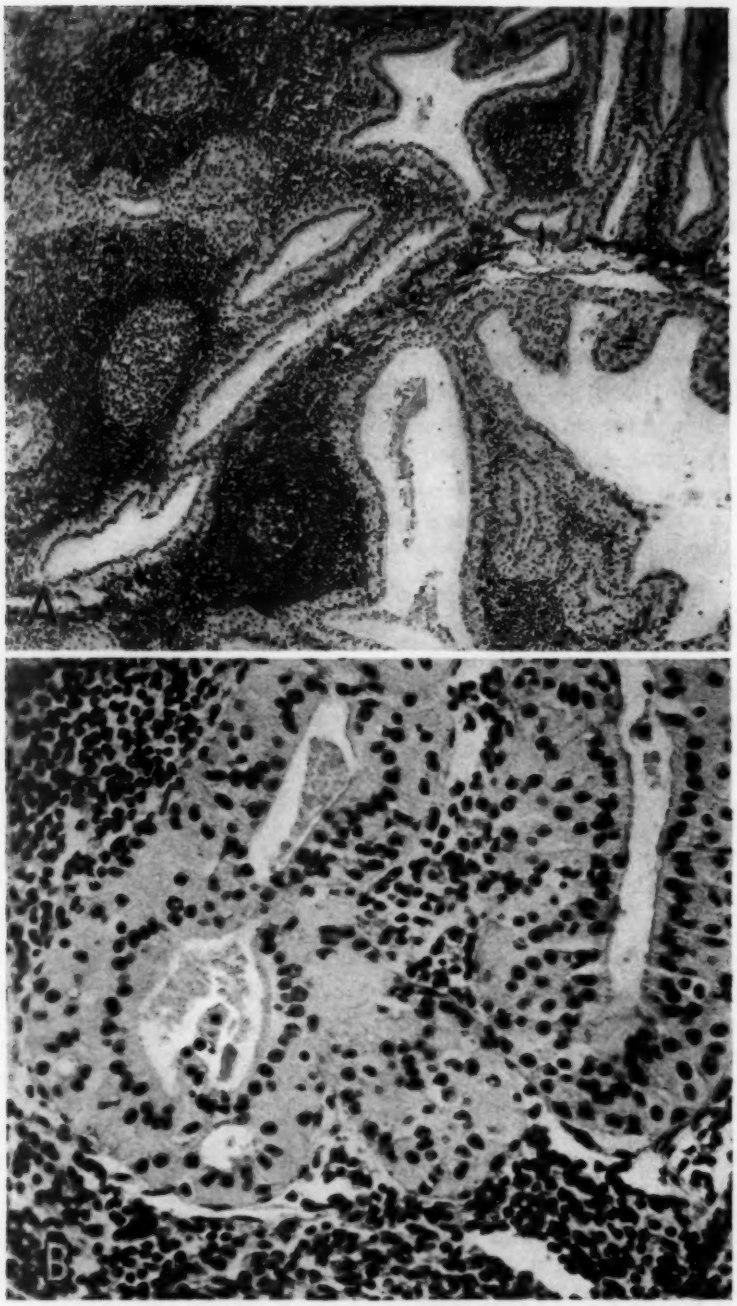

Fig. 1 (case 1).—*A*, lymphoid stroma. Hematoxylin and eosin; × 110. *B*, double rows of cell with nuclei near the lumen. Hematoxylin and eosin; × 440.

round cells with many lymph follicles, some of which contained well developed germinal centers. Near one end of the section there was a small portion of normal salivary gland with well defined acini and ducts. The whole was encapsulated by a narrow band of dense hyalinized fibrous connective tissue.

The diagnosis was papillary adenocystoma lymphomatosum of the parotid gland.

CASE 2.—A woman of 57 years entered the hospital Dec. 25, 1940 (service of Dr. D. Farber), complaining of a lump in the right cheek of five years' duration. Eight weeks prior to admission the mass became tender and began to enlarge rapidly. The tumor was firm, not tender, well demarcated and 7.5 by 5 cm. in size, with the posterior portion extending beyond the angle of the mandible. The skin was not attached, but the mass was apparently bound down to the deep fascia. It was removed, with the patient under general anesthesia, and the subsequent course in the hospital was uneventful.

The specimen was a firm nodular ovoid mass, which measured 4.2 by 2.3 by 2.1 cm. The external surface was pink-red and roughened by gray-yellow fibrous tags. In the cut surfaces could be seen gray-pink lobules separated by pearly gray strands. The central portion was soft and contained spaces up to 0.3 cm. in diameter.

Histologically, the tumor consisted of numerous irregularly shaped areas separated by broad bands of dense hyalinized fibrous connective tissue. Within these areas were numerous small irregular varying-sized lumens lined by two rows of columnar cells with abundant pink cytoplasm and deeply staining round or oval nuclei. In some places these cells were mounted on delicate branched papillary vascular connective tissue stalks. The stroma contained numerous lymph follicles, some with germinal centers. One end of the section showed a dense fibrous connective tissue capsule; the other end showed a small portion of salivary gland tissue with well defined acini and ducts. The interstitial connective tissue was densely infiltrated by small round cells.

The diagnosis was papillary adenocystoma lymphomatosum.

CASE 3.—A salesman of 45 years entered the hospital April 14, 1941 (service of Dr. D. Teplitsky), complaining of a swelling in the left cheek of one year's duration. This was not painful, but during the past year it had grown progressively larger. There was an egg-shaped mass of rubbery consistency, 6 by 3 cm., in front of and a little below the left ear. It was well encapsulated and not attached to skin, deep tissues or bone. There was no evidence of inflammation or fluctuation. It was removed, with the patient under general anesthesia. The patient made an uneventful recovery and was discharged April 18.

The specimen was a soft, roughly oval encapsulated mass, measuring 3 cm. in its greatest diameter. The surface was pink-gray, smooth and glistening. The cut surfaces were gritty, translucent and pink-gray. Within the center was a small cyst, 0.5 cm. in diameter, filled with blood, and near one end a larger cyst, 1 cm. in diameter, filled with thick yellow-green material.

Histologically, the tumor consisted of many delicate papillary stalks on a delicate thin basement membrane, on which were mounted uniform, tall, columnar cells containing pink cytoplasm and oval or round, deeply staining nuclei (fig. 2). The nuclei were situated near the lumens. In places the cells formed lumens, with infoldings of the epithelium in some, which were lined by similar cells.

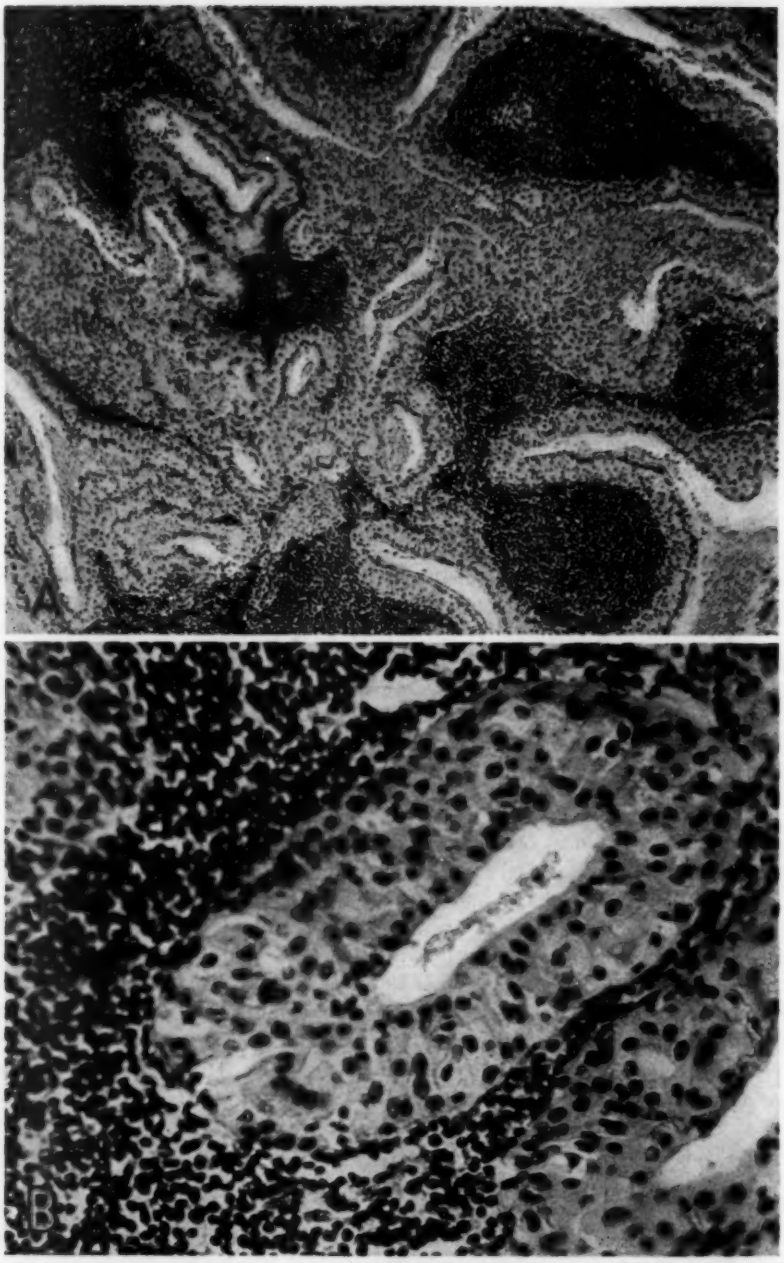

Fig. 2 (case 3).—Sections of tumor stained with hematoxylin and eosin; *A*, × 110; *B*, × 440.

The stroma contained many lymph follicles, in some of which were germinal centers. A capsule of hyalinized connective tissue covered part of the tumor.

The diagnosis was papillary adenocytoma lymphomatosum of the left parotid gland.

CASE 4.—A man aged 54 years entered the hospital (service of Dr. L. Morse) July 18, 1942. About four months before, he noticed that the left side of his cheek was raised. His physician advised removal of a mass.

At the angle of the left mandible, near the temporomandibular joint, a small tumor about 5 by 7.5 cm. was found. It was adherent to the underlying structures, but the skin over it was freely movable. It felt hard and smooth and was not painful or tender. Roentgen examinations showed no involvement of bone. The clinical diagnosis was mixed tumor of the parotid gland, bilateral indirect inguinal hernia, kyphosis and pulmonary emphysema. The tumor was removed on the next day, with the patient under general anesthesia. Except for a slight elevation of temperature and symptoms suggestive of acute cholecystitis with some pancreatic involvement, the patient made an uneventful recovery, the wound of the operation healing by primary union.

The specimen was a roughly oval-shaped encapsulated mass, measuring 3.5 by 2.5 by 1.6 cm. and weighing 11 Gm. (fig. 3*A*), removed from the left parotid region. On the external surface, lobulations could be recognized. On section the tumor mass was surrounded by a delicate thin transparent capsule. The cut surface presented a granular appearance, but no definite lobulations could be recognized. In a gray homogeneous background there were many small areas which were yellow or yellow with a greenish tinge. These varied in size up to about 2 mm. in diameter and were of irregular shape, many appearing only as yellow-gray strands. A few small hemorrhagic areas were scattered throughout the specimen.

On microscopic examination, the section showed a structural pattern composed of numerous irregularly shaped and sized lumens, with a lining in most instances of a single layer of extremely tall cells. In some places a double layer could be made out. With the hematoxylin-eosin stain, these cells showed distinct walls enclosing a bright red-staining, slightly granular cytoplasm. The nuclei were small, oval and vesicular, and situated close to the luminal poles of the cells. In some, distinct nucleoli were seen. Although for the most part these ductlike structures were lined by a single layer of cells, duplications and even heaping up, with spurlike formations, were occasionally encountered. The cells were mounted on a delicate stalk of a few fine fibrils of connective tissue, in which a capillary could usually be demonstrated. No broad arrangements like trabeculae could be found. Rarely a few nests of epithelial cells occurred. The contents of the lumens varied. In some there was a solid mass of pink-staining amorphous material; in others this was mixed with cellular and nuclear remains and debris, while in still others these contents contained boat-shaped clefts suggesting previous cholesterol deposits. The walls of these tubular structures devised an intricate maze of anastomosing, branching walls, which was strikingly emphasized by the strict uniformity of the cell pattern. Between them, and often separating them by broad areas were small round cells, again uniform in size, shape, appearance and staining characteristics. They were characterized by a relatively small amount of pale pink-staining cytoplasm and a definite cell membrane, with a large chromatic nucleus situated in the center. These resembled small lymphocytes in all respects (fig. 3*B*). They were

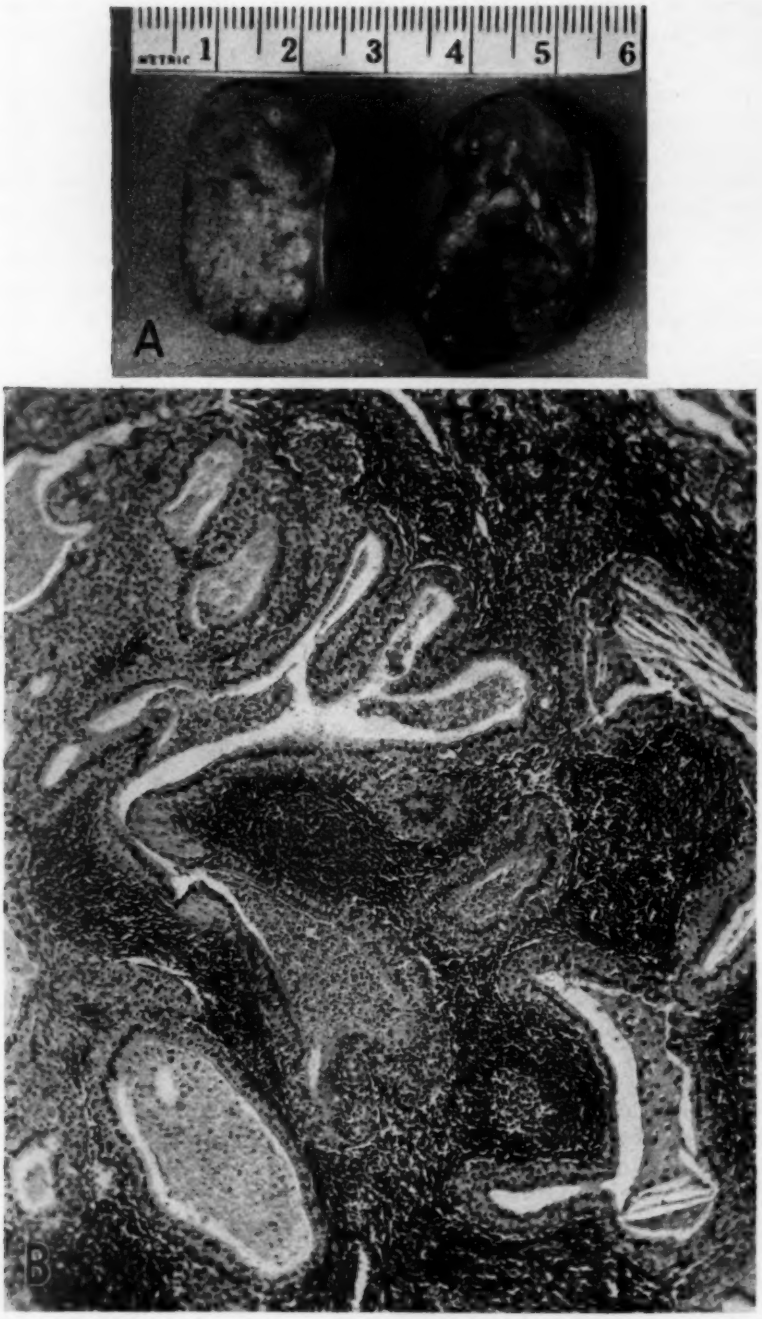

Fig. 3 (case 4).—*A*, gross appearance of tumor. *B*, section stained with hematoxylin and eosin; × 100.

also found in small numbers in the stalks that formed the framework of the walls of the tubules. In the larger collections, definite groups of endothelial cells characteristic of germinal centers were found. An occasional area of degeneration with hyaline changes was encountered.

The diagnosis was papillary cystadenoma lymphomatosum.

COMMENT

Papillary cystadenoma lymphomatosum is benign, grows slowly, and does not recur after complete removal. It is firm, encapsulated, lobulated. It is generally located on the external surface of the salivary glands, the most usual site being the parotid gland. It occurs commonly in adults past 40 years of age but has also been observed in children. The youngest patient reported was a boy of 2½ years and the oldest a man of 92 years.

Grossly, the tumor is purple-gray, ovoid or coarsely lobulated and usually from 2 to 6 cm. in the greatest diameter. It cuts with ease, and the cut surfaces are smooth or finely granular and may contain small cysts filled with serous or mucinous material.

Microscopically, the tumor is constructed of delicate branched papillary stalks, of tubular glands, ducts and small cysts and of a finely reticulated lymphoid stroma, in which there may be many follicles containing germinal centers. The lining cells are arranged in double rows occasionally in a single layer (case 4); they are tall columnar, with distinct cell borders, pale-staining cytoplasm and deeply staining nuclei, which are located near the lumen. The cells rest on a thin basement membrane and are arranged in two rows.

The name given to this tumor is merely descriptive of the histologic appearance and does not indicate the genesis. Of all the theories advanced, the most acceptable in our opinion is the theory that these tumors arise from heterotopic salivary gland epithelium enclosed in lymphoid tissue. Evidence that they originate from branchiogenic structures, from oncocytes or from aberrant anlages of the eustachian tube is not convincing. Their origin from orbital inclusion cysts would be more plausible if such cysts could be demonstrated in man. That salivary gland rests are occasionally found in cervical lymph nodes in the region of the salivary glands has been noted by several observers, and it is reasonable that such rests may on occasion develop into a tumor combining the structures of salivary gland ducts and lymphadenoid tissue. Although the tumor consists of this mixture of two distinct tissues, it bears no relation to the very common mixed tumors of salivary glands, although it is almost always clinically diagnosed as of that group.

SUMMARY

Four cases of papillary cystadenoma lymphomatosum are reported, and the literature is reviewed. The evidence at hand indicates the origin of this tumor in inclusions of salivary gland tissue in lymph nodes.

PANCREODOCHOCHOLECYSTOSTOMY AND EXPERIMENTAL PRODUCTION OF GALLSTONES

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Knowledge of the formation of gallstones in man is still in its infancy. This may be attributed to the following facts: First, stones vary greatly in composition (Phemister and co-workers [1]). Second, the methods of chemical analysis have not been perfected—some constituents are well known but others are more or less hypothetic. Third, there is a dearth of reports of analyses of human gallstones.

Animal experimentation has yielded some worth while results. Stones have been produced, but their resemblance to human gallstones has not been very close, and calcium has been either absent or low in amount. Various methods have been employed in the experimental production of gallstones: Concretions are mentioned as the result of infection of the gallbladders of dogs, rabbits or guinea pigs with either Bacillus typhosus or Bacillus coli by Gilbert,[2] Gilbert and Fournier [3] and Mignot [4]; Rosenow [5] obtained similar results, using streptococci in rabbits. These reports, however, include no description of the experiments.

A calcium carbonate precipitate in the gallbladder was produced by Wilkie [6] in 1928 by ligation of the cystic duct and injection of anhemolytic streptococci into the gallbladder. The same method was successfully applied and thoroughly studied by Phemister, Day and Hastings,[7] calcium carbonate concretions being produced in several cases.

There are only rare and inconsistent reports on concretions produced by feeding experiments. Thus the colloidal chemical theory advocated by Aschhoff, Bacmeister, Shade and others has found insufficient experimental support.

The only other method that has brought about a precipitate in bile in a series of cases is the introduction of foreign bodies into the gall-

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From the Department of Surgery, the University of Chicago.

This work was done in part on a grant from the Douglas-Smith Foundation for Medical Research.

1. Phemister, D. B.; Aronson, H. G., and Pepinsky, R.: Ann. Surg. **109**:161, 1939. Phemister, D. B., and Aronson, H. G.: Am. J. Path. **17**:673, 1941.
2. Gilbert, A.: Arch. gén. de méd. **10**:257, 1898.
3. Gilbert, A., and Fournier, L.: Compt. rend. Soc. de biol. **4**:936, 1897.
4. Mignot, R.: Arch. gén. de méd. **10**:129, 1898.
5. Rosenow, E. C.: J. Infect. Dis. **19**:527, 1916.
6. Wilkie, A. L.: Brit. J. Surg. **15**:450, 1928.
7. Phemister, D. B.; Day, L., and Hastings, A. B.: Ann. Surg. **96**:595, 1932.

bladder or bile ducts. Kretz,[8] in 1913, reported "stone formation" on threads left at operation in the gallbladders of rabbits and dogs, and I have observed dark incrustations on nonabsorbable suture material which was used for the closure of incisions in the gallbladder or in the common duct in dogs. These concretions resembled those obtained by Rous[9] and his co-workers, who described precipitation in a glass cannula inserted in the common duct and in a long rubber tube attached to the cannula and leading to a collecting bag outside the abdomen.

I[10] was able to obtain similar deposits on cannulas inserted in the common ducts of dogs. These concretions contained small quantities of calcium and pigment and a large amount of a residue not accounted for. In only 1 instance was I able to find stones—2 small ones lying free in a grossly noninflamed gallbladder in a dog whose common duct was slightly stenosed. The concretions contained cholesterol, calcium, some pigment, a trace of phosphorus and residue.

Recently another experimental approach has been resorted to. It has been learned from postmortem and operative observations and also from the experiments of Dragstedt[11] and co-workers that reflux of bile into the pancreatic duct may cause necrosis of the pancreas. The possibility of the reversed process, i. e., reflux of pancreatic juice into the gallbladder with subsequent damage to the gallbladder wall, has been suggested by Wolfer.[12] This author collected clinical cases from the literature and reported several of his own in which obstruction of the sphincter of Oddi, cholecystitis and bile peritonitis were present. From the presence of pancreatic ferments in the bile of both the biliary system and the abdominal cavity, it was concluded that reflux of pancreatic juice into the gallbladder had caused these changes.

Bisgard and Baker[13] carried this idea further in their experiments on goats, in which the pancreatic duct opens into the common duct above the ampulla of Vater. When he ligated the common duct above the entrance of the pancreatic duct, the changes in the gallbladder were moderately severe. On the other hand, when the ligation was done distal to the entrance of the pancreatic duct, permitting reflux of pancreatic juice, the gallbladder wall became necrotic.

Bisgard and Baker found stones in three of their experiments. In these cases the obstruction distal to the pancreatic duct was maintained for from ninety-six hours to six days, and the animals were killed from two and one-half to six months later. The stones were composed of bile pigment. "They were numerous and very small, and were present

8. Kretz, R., in Krehl, L., and Marchand, F.: Handbuch der allgemeinen Pathologie, Leipzig, S. Hirzel, 1913, vol. 2, pt. 2, p. 493.

9. Rous, P., and McMaster, P. D.: J. Exper. Med. **37**:11, 1923.

10. Aronson, H. G.: Arch. Surg. **41**:960, 1940.

11. Dragstedt, L. R.; Haymond, H. E., and Ellis, J. C.: Arch. Surg. **28**:232, 1934.

12. Wolfer, J. A.: Surgery **1**:928, 1937.

13. Bisgard, J. D., and Baker, C. P.: Ann. Surg. **112**:1006, 1940.

in both the gall bladder and the dilated common duct. The other gall bladder contained 3 stones, 2 of which were very small and one fairly large."

EXPERIMENTAL STUDIES

In order to study further the relationship between the gallbladder and the pancreas I decided to make an anastomosis between the pancreatic duct and the gallbladder. Dogs were selected for our experiments because stones are rarely if ever formed in them and reflux of pancreatic juice into the gallbladder under normal conditions is unlikely for anatomic reasons. The common duct and the pancreatic duct enter the duodenum separately at a distance of about 1 inch (2.5 cm.) from each other.

Procedure.—With the dog under ether anesthesia and with strictly aseptic conditions, the abdomen was opened by a midline incision and the peritoneum between the pancreas and the duodenum incised on either side. The main pancreatic duct was dissected, and the blood vessels were ligated proximal and distal to the duct at a distance of about 1 cm. The duct was excised from the duodenum with a small piece of duodenal wall (about 0.5 cm. in diameter), and the opening in the duodenum was closed. Bile was aspirated from the gallbladder and an incision made on the lower surface of the fundus. Through this opening the excised piece of duodenum with the duct was pulled inside the gallbladder by means of two sutures, which were carried through the opposing upper surface and tied. The pancreas then was sewed to the serosal lining of the gallbladder around the duct and the area of operation covered with omentum.

Four dogs in all were operated on. All the animals recovered from the operation quickly and remained in good physical condition. They were kept on a general mixed diet. No jaundice or other abnormalities were noticed. The 4 dogs were killed after two years. In 3 animals it was found that the anastomosis was tightly closed by scar formation. The biliary system and the pancreas appeared normal on gross and on microscopic examination, and one can assume that sufficient accessory ducts had been present to prevent stasis in the pancreas. In 1 dog, however, a well functioning anastomosis was found, and stones had formed in the gallbladder which closely resembled human gallstones. Therefore this experiment will be described more fully.

Protocol.—Dog 175, weighing 14 pounds (6.5 Kg.), was submitted to pancreodochocholecystostomy June 15, 1939 and was killed Oct. 7, 1941 (fig. 1).

Post mortem, extensive adhesions were found between the gallbladder, the pancreas and the duodenum. The gallbladder was dilated to about the size of a hen's egg. The pancreas was markedly atrophic and nodular. The pancreatic duct was considerably dilated and connected with the gallbladder through a wide open anastomosis.

The gallbladder contained 25 cc. of grayish brown cloudy fluid and three round hard dark brown concretions the surfaces of which were covered with strawberry-like small dots. Two of these concretions were about the size of a pea, while the third one was slightly larger. In one of the smaller stones a silk suture was embedded with the ends of the suture protruding from the surface of the stone. The suture was removed from the stone without disturbing its shape. The gallbladder wall appeared grossly normal.

Cultures of the bile revealed Staphylococcus aureus and Clostridium welchii.

Microscopic examination showed slight diffuse and focal leukocytic infiltration of all layers of the gallbladder. In the pancreas (fig. 2) there were some areas of

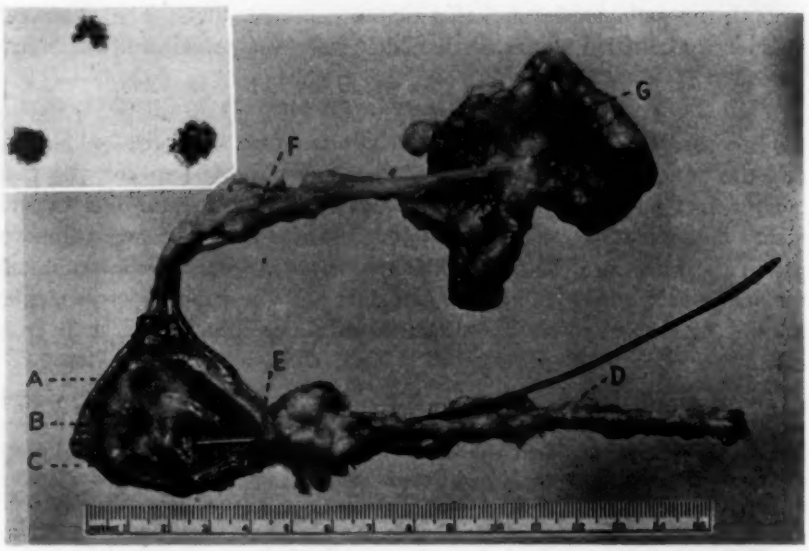

Fig. 1.—Pancreodochocholecystostomy was performed two years ago. *A*, *B*, *C* indicate the stones found in the gallbladder (*G*); *D*, the pancreas with the pancreatic duct partially opened; a probe is passed through the anastomosis (*E*) into the gallbladder. *F* indicates the bile duct. The insert is a roentgenogram of stones casting dense calcium shadows.

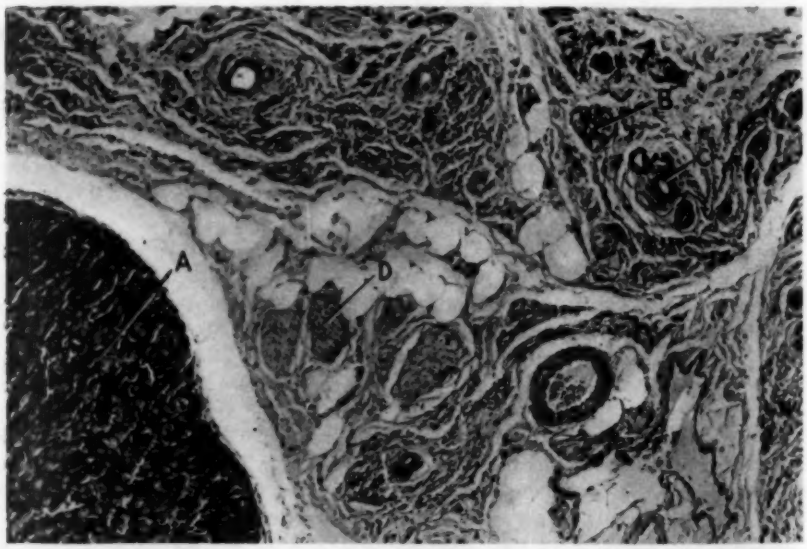

Fig. 2.—Photomicrograph of pancreas: *A*, normal pancreatic tissue; *B*, islands; *C*, ducts, and *D*, nerves within atrophic acinous tissue.

normal pancreatic tissue, but the acinous tissue was atrophic in many places. A moderate number of ducts and islands persisted, and acinous tissue was replaced by fibrosis.

Chemical Analysis.—The weights of the stones, A, B and C, were 0.090 Gm., 0.040 Gm. and 0.038 Gm., respectively. Stones A and C were analyzed together, while B, which contained the suture, was analyzed separately.

	A + C	B
Ether extraction (by weight)	5.6 %	5.8 %
Pure cholesterol (by colorimeter)	0.4 %	0.3 %
Calcium	23.0 %	28.6 %
Phosphorus	0.06%	0.1 %
Pigments	0.1 %	0.04%

COMMENT

From the postmortem observations one may assume that there was mixing of pancreatic juice with bile for a long period. The pancreatic juice must have been considerably diluted by bile since the gallbladder wall had not been attacked by the juice and had remained practically normal. After two years there were no signs of edema or necrosis, which Wolfer described as characteristic changes following the recent injection of pancreatic juice into the gallbladder.

Infection seems to have played a minor part. Cultures of the bile revealed growth only of organisms frequently found in bile, and the changes in the gallbladder wall were slight.

Some stasis in the gallbladder may have been present, since it was dilated. The bile ducts, however, were of normal width, and the increase in pressure may have been caused by the added flow of pancreatic juice. As Dragstedt has shown, the pressure in the pancreatic system during the height of digestion is slightly higher than that in the biliary, and it may well be that the combined pressures caused the dilatation of the gallbladder.

On the other hand, there is no definite explanation for the atrophy of the pancreas. The gross and microscopic appearances of the pancreas are similar to those which were described by Soboleff [14] in 1900 in a case of obstruction of the pancreatic duct; i. e., there was atrophy of acinous tissue with persistence of islets and increase of interstitial tissue. There are two possible explanations for this obstruction. First, it was due to temporary edema about the anastomosis following the operation; second, it was due to increased pressure in the gallbladder. However, another factor to be considered is the disturbance of the blood supply by the ligation of blood vessels during operation.

It is of interest that the experimentally produced stones resemble those generally found in the pancreatic duct of man in that both contain much calcium and are similar in shape. A description of human

14. Soboleff, L. V.: Centralbl. f. allg. Path. u. path. Anat. **11**:202, 1900.

gallstones that might be called typical was given in 1914 by Rosenthal,[15] who found stones in the pancreatic duct which were almost pure calcium carbonate and had strawberry-like surfaces.

In connection with this one ought to consider the possibility that the stones in the experiment described in this report had formed in the pancreatic duct and were later expelled into the gallbladder.

I am unable to make any definite statement as to the mechanism of stone formation. Since part of a thread was incorporated in one of the stones, one might consider this stone to be the result of a foreign body reaction; however, I do not believe that this was the case, because the thread was located on the periphery of the smallest stone and could be easily removed without disturbing the shape of the stone. The uniformity of the results of chemical analysis of all three stones, two of which were free of any foreign body, tends to indicate that their formation was probably due to the same mechanism.

I conclude from the experiments described that the alkaline pancreatic juice, aided by some stasis, was influential in causing the formation of gallstones rich in calcium.

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15. Rosenthal: Arch. f. Verdauungskr. **20**:619, 1914.

SUDDEN DEATH FOLLOWING INJECTION OF FOREIGN PROTEIN

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AND
GEORGE STRASSMANN, M.D.
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The injection of an antitoxin or other foreign protein for prophylactic or therapeutic purposes has been followed in rare instances by the collapse and death of the patient. Park [1] has estimated that such a death occurs about once in every seventy thousand injections. In a few of such cases necropsies have been made, but the published reports have yielded only a limited amount of information. Up to the present the results of these investigations have not been sufficiently comprehensive to provide a proper basis for a suitable classification of the available evidence.

We have collected the reports of seven necropsies in the Office of the Chief Medical Examiner of the City of New York in cases of sudden death following the injection of foreign protein. These cases have been correlated with 19 similar cases reported in the literature, in which necropsies were made. In considering this material the most convenient method has been to separate the cases into known asthmatic cases and presumably nonasthmatic cases.

SUDDEN DEATH FOLLOWING PROPHYLACTIC OR THERAPEUTIC INJECTION OF ANTITOXIN IN PRESUMABLY NONASTHMATIC CASES

CASE 1.—A white girl aged 3½ years received a prophylactic injection of diphtheria antitoxin (1,000 units) and five minutes later became cyanotic and dyspneic. On admission to a hospital one-half hour later, she showed infrequent and spasmodic respirations, only two to three a minute, with palpable and audible wheezing and strenuous use of the accessory respiratory muscles; the chest was inflated, and rhonchi were audible on auscultation. The face was cyanotic, the jaws were tightly clenched, the limbs were cold and clammy to the touch, the pulse was weak and rapid, and there was incontinence of urine and feces. Lateral nystagmus was observed.

In regard to her previous medical history the father stated that when the patient was a baby she had been given three injections of some unknown substance at a clinic. There was no history of bronchial asthma.

From the Office of the Chief Medical Examiner of the City of New York and the Department of Forensic Medicine of New York University.

1. Park, W. H.: J. A. M. A. **76**:109, 1921.

The patient was immediately given epinephrine hydrochloride by intracardiac injection, and her condition improved. One-half hour after admission the respiratory rate was 36 per minute, the pulse rate was 148 per minute and the temperature was 97 F. One hour after admission a second attack came on, similar to the first, with distention of the chest and labored infrequent respirations; this was relieved by the same therapy. Four subsequent attacks followed at about hourly intervals and were treated by the administration of epinephrine and occasionally

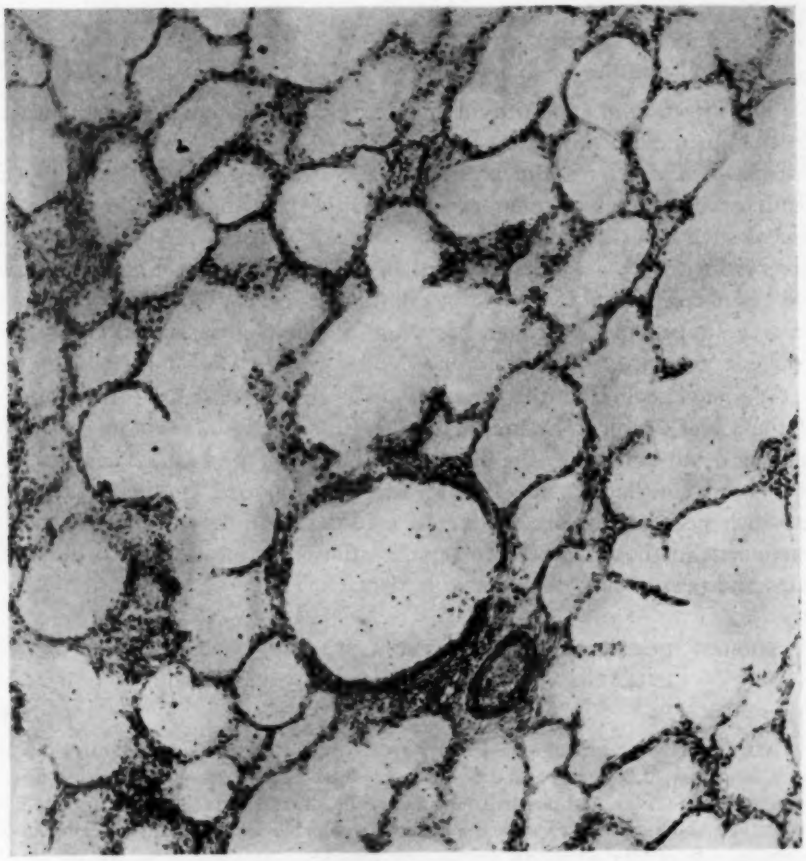

Fig. 1 (case 1).—Marked inflation of alveoli and a bronchiole in lung tissues; low power magnification.

by artificial respiration and the application of external heat. The patient finally became unconscious and died six hours after the injection of the antitoxin.

Necropsy (fifteen hours after death).—The body was that of a white girl 38 inches (96.5 cm.) in height and 32 pounds (14.5 Kg.) in weight, well formed and well nourished. The skin was smooth and fine in texture. Many hypodermic needle punctures were present on the arms, and two were noted over the cardiac area. The chest was fixed in full inspiration.

The lungs were distended, pale and well aerated. Occasional small irregular depressed areas of dark red atelectasis were found on the posterior surfaces of both lungs. The bronchi were pale, contained a little mucus but otherwise were apparently normal. There was no edema of the submucous layer of the larynx. Both the larynx and the trachea appeared natural.

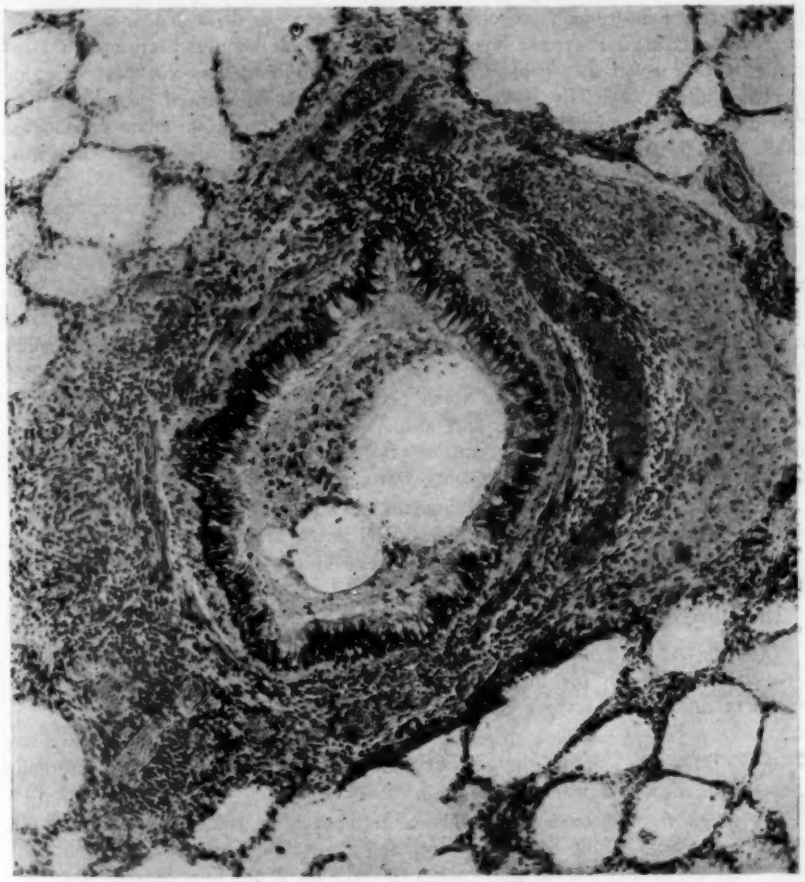

Fig. 2 (case 1).—Small bronchus showing mucus in the lumen, the epithelial layer thrown into projecting folds, congested blood vessels and cellular infiltration of the wall; low power magnification.

The heart was normal and contained postmortem red and yellow clot and fluid blood. A few minute longitudinal flamelike subendocardial hemorrhages were present on the septal portion of the left ventricle.

The liver, the kidneys and the brain were moderately congested. The adrenals were small but otherwise natural. The lymphoid tissue in the intestines and the spleen was about normal in amount. The thymus was large, weighing 45 Gm., and was composed entirely of lymphoid tissue.

Microscopic Examination.—The tissues were embedded in a shredded collodion (paralodion) and stained with hematoxylin and eosin.

In the lungs, most of the alveoli were markedly distended and thin walled; their capillaries were small and contained only a few polymorphonuclear neutrophilic leukocytes. In certain areas near the terminal bronchioles there were groups of alveoli which were not expanded and in which the capillaries contained some polymorphonuclear neutrophilic leukocytes and a few eosinophilic leukocytes. The smaller bronchioles which lacked muscular coats were distended (fig. 1). The bronchi possessing a muscular layer showed the muscle bundles contracted, the epithelial layer thrown into projecting folds and considerable mucus present in the bronchial lumen (fig. 2). Both large and small bronchi showed submucous infiltration by large mononuclear cells, neutrophilic and eosinophilic polymorphonuclear leukocytes and a few plasma cells; the eosinophilic leukocytes were more numerous in the larger than in the smaller bronchi. The smaller bronchial blood vessels were engorged, containing many neutrophilic and eosinophilic polymorphonuclear leukocytes as well as red blood cells.

The submucous layer of the larynx near the true vocal cords contained a submucous infiltration of the same types of cells already described in the bronchial wall.

In a bronchial lymph node the germinal centers were large and contained large reticulum cells filled with nuclear fragments. A large number of eosinophilic leukocytes were present in the sinuses of the node.

Many eosinophilic leukocytes were present in the submucous layer of the duodenum, ileum and appendix, and a moderate number were seen in the submucous layer of the stomach. Sections of the appendix disclosed a cross section of a small worm in the lumen, probably an oxyuris.

Large numbers of eosinophilic leukocytes were present in the splenic pulp. The splenic follicles were similar to those described in the bronchial lymph node.

A few neutrophilic leukocytes were present in the sinusoids of the liver, but the organ was otherwise normal.

The other organs were normal.

Case 2.—A white boy aged 4 years and 9 months received a subcutaneous injection of antitoxin for incipient scarlet fever and died a few minutes later. Details of his medical history could not be obtained.

Necropsy (about twelve hours after death).—The body was that of a white boy 45 inches (114 cm.) in height and 40 pounds (18 Kg.) in weight. Externally there was no sign of a scarlet fever rash.

The lungs were distended and did not collapse after being removed from the body. There was slight edema present, and the bronchi contained some frothy fluid. The heart was normal and contained some dark red fluid blood. The liver and the kidneys were normal but markedly congested. The brain was swollen and heavy and showed considerable cerebral edema. The tonsils were enlarged, and the crypts contained some yellowish white material. The lymphoid tissues of the spleen, the lymph nodes, the base of the tongue and the intestines were definitely hypertrophic. The thymus was composed of lymphoid tissue and weighed 50 Gm.

Microscopic Examination.—The only difference between the bronchi and lungs in this case and the same organs in case 1 was that they showed less distention of the alveoli and only a few eosinophilic leukocytes in the submucous layer of the bronchi. The tonsils showed a slight subacute inflammatory reaction around the necrotic material in the crypts. There was a moderate number of neutrophilic leukocytes in the sinusoids of the liver. The lymphoid tissue generally contained

large germinal centers with numerous reticulum cells, but phagocytosis was lacking. The brain tissue was normal aside from distention of the Virchow-Robin spaces by fluid. The other organs were normal.

CASE 3.—A white girl aged 10 years received a superficial wound in the back from the discharge of a blank cartridge pistol. She was given 750 units of tetanus antitoxin intramuscularly as a prophylactic measure and half an hour later was admitted to a hospital in coma. She died fourteen hours after the injection. Further details of her medical history could not be obtained.

Necropsy (about twenty-four hours after death).—The body was that of a white girl 4 feet 11 inches (150 cm.) in height and 105 pounds (47.5 Kg.) in weight, well developed and well nourished. The face and neck were markedly cyanotic. There was a small circular wound, ¼ inch (0.6 cm.) in diameter, in the back of the left side of the chest; this barely penetrated the dermis, was not inflamed and was produced by the discharge of a blank cartridge. Numerous hypodermic needle puncture marks were present over both arms.

The lungs were distended but at the same time were congested. A few small dark red depressed areas of atelectasis were present here and there on the surface. Thick, tenacious mucus was present in the trachea and the bronchi. There was considerable edema of the submucous layer of the larynx near the vocal cords, but not sufficient to obstruct the air passages. The liver and the kidneys were markedly congested. The brain was considerably congested and swollen to such an extent that it was under compression against the dura; the pons was flattened, and the cerebellum was indented by its contact with the foramen magnum. The thymus weighed 20 Gm. and was composed of lymphoid tissue. The other lymphoid tissues were moderately hyperplastic.

Microscopic Examination.—The lungs and the larynx showed the same general characters noted in case 1 except that eosinophilic leukocytes were present only in moderate numbers in the submucous layer of the larynx and bronchi. The other organs were not examined microscopically.

CASE 4.—A white boy of 11 years was given subcutaneously a prophylactic injection of 1,500 units of tetanus antitoxin for a laceration of the left hand. He immediately went into collapse and died in about three minutes. Further details of his medical history could not be obtained.

Necropsy (about sixteen hours post mortem).—The body was that of a well developed white boy 54 inches (137 cm.) in height and 64 pounds (29 Kg.) in weight. A laceration ¾ inch (2 cm.) long and ⅜ inch (1 cm.) deep was found in the palm of the left hand. Numerous hypodermic needle puncture marks were found over both arms. The tongue was tightly clenched between the teeth.

The lungs were well aerated and feathery in consistency, and there was moderate hypostatic congestion posteriorly. The heart contained fluid blood, and flamelike subendocardial hemorrhages were visible on the septal surface of the left ventricle. The lymphatic tissues were hyperplastic over the body. The thymus was composed of lymphatic tissue and weighed 42 Gm. There was distinct edema of the loose submucous tissues at the base of the epiglottis, extending down to the vocal cords, but not of sufficient intensity to obstruct the larynx. The brain, the liver and the kidneys were congested but normal. The adrenals were small but normal. A microscopic examination was not done in this case.

CASE 5.—A white girl aged 16 years was given a prophylactic injection of 1,500 units of tetanus antitoxin subcutaneously for a laceration of the right foot. She

suddenly collapsed one-half hour later and died in a few minutes. There was no history of a previous injection of horse serum nor was there any history of bronchial asthma.

Necropsy (about twenty-four hours after death).—The body was that of an adolescent white girl 5 feet 1 inch (155 cm.) in height and 125 pounds (56.5 Kg.) in weight, well formed and well developed. On the dorsum of the right foot was a puncture wound 1/16 inch (0.16 cm.) in diameter and 3/8 inch (1 cm.) deep, apparently of recent origin. On the right forearm there was a hypodermic needle puncture which was surrounded by a distinct zone of subcutaneous edema.

The chest was markedly distended. The lungs were aerated, pale and inflated to such an extent that they covered the pericardium. A few pinhead-sized subpleural hemorrhages were present in the interlobar fissures. The air passages contained a little thick mucus but were otherwise normal. The lymphatic tissues throughout the body were moderately hyperplastic. The thymus was composed of lymphatic tissue, and weighed 12 Gm.

Microscopic Examination.—The lung tissue showed distended alveoli, and there was an infiltrate of eosinophilic leukocytes and other cells in the submucous layer of the bronchi as described in case 1. Numerous eosinophilic leukocytes were found in the submucous layer of the stomach and the sinusoids of the spleen, and a few were present in the sinusoids of the liver.

SUDDEN DEATH FOLLOWING INJECTION OF FOREIGN PROTEIN EXTRACTS IN CASES OF BRONCHIAL ASTHMA

CASE 6.—A Negro girl aged 4 years, known to be asthmatic, was given an intracutaneous test injection of a mixture of silkworm, sheep's wool and kapok. Soon after this she went into shock, with symptoms of unconsciousness and cyanosis, and died in about three hours.

Necropsy (eighteen hours after death).—The body was that of a Negro girl 40 inches (101.5 cm.) in height and about 35 pounds (16 Kg.) in weight. The lungs were markedly distended and mottled bluish gray and reddish gray. The bronchi and the trachea were congested and their walls thickened. The heart contained fluid blood and was normal aside from a few pinhead-sized subepicardial hemorrhages. The liver, the spleen, the kidneys and the brain were congested. There was hyperplasia of the lymphatic tissues of the body. The thymus was large and lymphoid, weighing 35 Gm.

Microscopic Examination.—The lung showed the same microscopic characteristics which were described in case 1, and large numbers of eosinophilic leukocytes were present in the submucous layer of the bronchi. In many places the smaller bronchi were plugged by a mucous secretion. The other organs were normal.

CASE 7.—A Negro man aged 35 years had been treated for bronchial asthma by repeated injections of ragweed pollen extract. He was found dead in the physician's office one hour after he had received his last injection.

Necropsy (about sixteen hours post mortem).—The examination was confined to the lungs and the heart. The body was that of a powerfully developed Negro man about 5 feet 9 inches (175 cm.) in height. The eyeballs were prominent, and the chest was distinctly expanded. The lungs were inflated so that they practically covered the precordium. The bronchi were thick walled and congested. The heart was normal and contained fluid blood.

Microscopic Examination.—The alveoli of the lungs appeared to be distended, and the submucous layer of the bronchi contained numerous eosinophilic leukocytes and other cells as described in case 1. In addition there was thickening of the basement membrane of the larger bronchi, and in places, metaplasia of the columnar epithelium to stratified squamous epithelium. Some of the connective tissue surrounding the cartilaginous plates of one of the smaller bronchi showed transformation into bony tissue.

The 7 cases were grouped as presumably nonasthmatic cases and as asthmatic cases.

Nonasthmatic Cases.—There were 5 cases in this group. All of the patients died after receiving by injection some derivative of horse serum: in case 1, diphtheria antitoxin; in cases 3, 4 and 5, tetanus antitoxin, and in case 2, scarlet fever antitoxin.

The clinical histories disclosed that in cases 2 and 4 the patients collapsed shortly after the injection of the serum and died in a few minutes. In case 1 the patient became markedly dyspneic about five minutes after the injection of the antitoxin but died after six hours of severe expiratory dyspnea. In cases 3 and 5 the symptoms started about half an hour after the injection; in case 5 the patient died in a few minutes of shock, but in case 3 the patient lived for fourteen hours, presenting unconsciousness as the most prominent symptom.

In all the cases the lungs were found to be markedly distended and the parenchymatous viscera were congested to a varying degree. In case 5 there was a slight subcutaneous edema around the site where the fatal dose of diphtheria antitoxin was injected. Cases 3 and 4 were noteworthy in that there was some submucous edema at the upper end of the larynx. The brain was edematous in cases 2 and 3, resulting in cerebral compression; microscopic examination of the brain in case 2 demonstrated that the Virchow-Robin spaces were distended with fluid. In all 5 cases there were marked hyperplasia of the lymphatic tissues and enlargement of the thymus. In cases 1, 4, and 5 there were subendocardial hemorrhages on the septum of the left ventricle, which were probably due to the excessive action of the heart during the final attack of asphyxia.

The microscopic examination of the lungs in cases 1, 2, 3 and 5 revealed marked distention of the alveoli and a cellular infiltration of the submucous layer of the bronchi in which eosinophilic leukocytes were taking part in greater or less numbers. The extent of the infiltration was greater, and the eosinophilic leukocytes present were more numerous, in the bronchial wall in our series of cases than in normal persons.

A pinworm was found in the appendix in case 1, but it could not be determined whether or not its presence had any influence on the clinical behavior of the patient.

Asthmatic Cases.—The 2 asthmatic patients died shortly after the injection in asthmatic attacks. The patient in case 6 received an injection of a mixed extract of sheep's wool, silkworm and kapok; in case 7 the patient was given an injection of ragweed pollen extract. In both there was distention of the alveoli with submucous infiltration of the bronchi by eosinophilic leukocytes and other cells as described in the series of presumably nonasthmatic cases. In case 7 there were in addition thickening of the basement membrane of the larger bronchi, metaplasia of the bronchial epithelium and in places transformation of some of the fibrous tissue around the bronchial cartilaginous plates into bone. The histologic lesions in case 7 were similar to some of the lesions described by Macdonald.[2]

The resemblance in our series between the presumably nonasthmatic and the known asthmatic cases is quite striking, as both present similar symptoms and similar pathologic changes. It is evident that after the injection of an antitoxin or other foreign protein some substance in the injection fluid causes a spasm of the bronchial muscles so that marked expiratory dyspnea and death by asphyxia result. In both types of cases the infiltrating eosinophilic leukocytes in the submucous layer of the bronchi suggest a long-standing inflammatory reaction against some irritant. Obviously the exudates in the different cases were chronic in type and were not evoked by the injection of foreign protein a few minutes or a few hours prior to death.

The laryngeal and the cerebral edema noted in some of the cases in part were doubtless produced by the circulatory disturbance following the acute distention of the lungs and in part were referable to the action of the antigen on the endothelial cells of the capillaries, which in these organs were rendered abnormally permeable to the fluid constituents of the blood. Moon [3] and Vaughan [4] mentioned this influence of antigen on capillary endothelium as an important part of the phenomenon of allergic shock.

A review of the literature discloses 19 cases in which death following an injection of horse serum or other foreign protein was investigated by necropsy. The presumably nonasthmatic cases are listed in table 1 and the known asthmatic cases in table 2. Our cases are also included in the appropriate tables: cases 1, 2, 3, 4 and 5 in table 1 and cases 6 and 7 in table 2.

An analysis of table 1 discloses that of the 18 patients whose cases are listed, 16 were white and 2 were Negroes. Nine of the patients were males and 9 were females. The patients fall into three age cate-

2. Macdonald, I. G.: Ann. Int. Med. **6**:253, 1932.

3. Moon, V. H.: Ann. Int. Med. **12**:205, 1938.

4. Vaughan, W. T.: Practice of Allergy, St. Louis, C. V. Mosby Company, 1939, pp. 14 and 74.

gories: 2 to 11 years—10 patients; 16 to 22 years—5, and 30 to 39 years—3; thus about 55 per cent were 11 years old or younger, and 83 per cent were under the age of 25 years.

Six of the 18 patients had received injections of antitoxin or some other foreign protein for periods of six to fourteen days to a number of years prior to death, while the remaining 12 had not received such injections. Two patients gave a previous medical history of symptoms which might be interpreted as signs of allergic sensitiveness; thus the patient of McCallum [5] had complained of repeated bronchial colds, and the patient of Gurd and Emrys-Roberts [6] reported periodic attacks of vomiting and diarrhea for a number of years prior to death.

The product injected immediately preceding the onset of the fatal symptoms was some derivative of horse serum in 17 cases and a typhoid vaccine in 1 case. While the dosage was mentioned in all except 1 case, it is difficult to appraise its significance, as the susceptibility of the patient was not ascertainable and some of them were desperately ill of an infectious disease at the time of the injection. In general, however, the greater the quantity of protein administered, the more rapid and severe was its action. The most violent effects were noted in the cases of Koch [7] and of Bullowa and Jacobi,[8] in which the serum was injected intravenously. Koch's case was interesting in that the patient had had an injection of diphtheria antitoxin two weeks prior to death and on the fatal day had been given a subcutaneous desensitizing injection of 5 cc. of antistreptococcus serum, followed five hours later by an intravenous injection of 10 cc. of antistreptococcus serum, which caused death; the desensitizing dose evidently had failed in its purpose.

Ten patients, of whom 7 were children and 3 were adolescents, began to breathe with difficulty soon after the injection and died within ten minutes. Death was due to asphyxia caused by muscular spasm of the smaller bronchi, as indicated by the clinical history or by the distended condition of the lungs at necropsy. The reports of 3 cases, namely, those of Klotz,[9] Dogge [10] and Kortright,[11] furnished so little information that it was not possible to determine much from them besides the fact that each patient died rapidly and dramatically soon after receiving an injection.

In 4 cases death occurred in twenty to seventy minutes, the symptoms starting soon after the injection or after an interval of twenty to thirty

5. McCallum, A. D.: Brit. M. J. **2**:596, 1919.
6. Gurd, F. B., and Emrys-Roberts, E.: Lancet **1**:763, 1920.
7. Koch, W.: Berl. klin. Wchnschr. **52**:685, 1915.
8. Bullowa, J. G. M., and Jacobi, M.: Arch. Int. Med. **46**:306, 1930.
9. Klotz, O.: Montreal M. J. **36**:615, 1907.
10. Dogge, C.: Pediatrics **2**:12, 1896.
11. Kortright, J.: Brooklyn M. J. **10**:86, 1896.

TABLE 1.—*Deaths Following Injection of Foreign Protein: Presumably Nonasthmatic Cases*

Author	Sex, Race and Age	Previous Injection of Foreign Protein	Fatal Injection: * Nature, Purpose, Amount	Interval Between Injection and Death	Clinical and Pathologic Findings
Klotz [9]	♂ White 2 yr.	None	Diphtheria antitoxin Prophylaxis 1.2 cc. in muscle	10 min.	Enlarged thymus at necropsy
Dogge [10]	♂ White 3 yr.	None	Diphtheria antitoxin Treatment 10 cc.	10 min.	Inflamed throat; no changes at necropsy
Vance and Strassmann, case 1	♀ White 3 yr., 6 mo.	Possibly given injections as a baby	Diphtheria antitoxin Prophylaxis 1,000 units	6 hr.	Symptoms began in 10 min.; difficult breathing, distended lungs
Vance and Strassmann, case 2	♂ White 4 yr., 9 mo.	None	Scarlet fever antitoxin Therapy Dose (?)	Few min.	Difficult breathing, distended lungs
Koch [7]	♀ White 6 yr.	Injection diphtheria antitoxin 2 weeks ago	Antistreptococcus serum Treatment 10 cc. in vein	2 min.	Septic throat, inflated chest, distended lungs
Ferguson [12]	♂ White 6 yr.	None	Tetanus antitoxin Prophylaxis for slight injuries Dose: 3 minims intradermally	2 hr.	Symptoms began in 15 min.; chest rigid, difficult breathing, lungs serosanguineous
McCallum [5]	♂ White 8 yr.	None	Diphtheria antitoxin Prophylaxis 2,000 units	5 min.	Choking, cyanosis, fluid blood in heart, lungs normal
Bullowa and Jacobi [3]	♀ White 8 yr.	None	Diphtheria antitoxin Treatment 5,000 units intravenously	Few min.	Diphtheritic throat, difficult breathing, inflated lungs
Vance and Strassmann, case 3	♀ White 10 yr.	None	Tetanus antitoxin Prophylaxis for blank cartridge wound 750 units in muscle	14 hr.	Coma began in 30 min.; distended lungs, edema of brain and larynx
Vance and Strassmann, case 4	♂ White 11 yr.	None	Tetanus antitoxin Prophylaxis for wound of hand 1,500 units	Few min.	Collapse, inflated lungs, edema of larynx
Kortright [11]	♀ White 16 yr.	None	Diphtheria antitoxin Treatment 10 cc. in muscle	8 min.	Convulsions; congestion of organs, lungs normal
Vance and Strassmann, case 5	♀ White 16 yr.	None	Tetanus antitoxin Prophylaxis for a wound of the foot 1500 units	30 min.	Symptoms in 30 min.; sudden collapse, distended lungs
Lamson [17] case 2	♀ White 17 yr.	None	Diphtheria antitoxin Prophylaxis 500 units in muscle	10 min.	Distended lungs, enlarged thymus
Waugh [21]	♀ White 17 yr.	Diphtheria antitoxin 10 yr. ago	Diphtheria antitoxin Treatment 4,000 units	10 min.	Membrane on tonsils, difficult breathing, congested lungs
Dean [13]	♂ White 22 yr.	Tetanus antitoxin, twice 1 mo. ago	Tetanus antitoxin Prophylaxis for war wound 500 units	70 min.	Symptoms at once; difficult breathing, distended lungs, cyanosis
Gurd Emrys-Roberts [6]	♂ White 30 yr.	None	Tetanus antitoxin Prophylaxis for war wound 750 units	23 hr.	Symptoms in 2 hr.; vomiting, diarrhea, then dyspnea, distended lungs, engorged liver

TABLE 1.—*Deaths Following Injection of Foreign Protein: Presumably Nonasthmatic Cases—Continued*

Author	Sex, Race and Age	Previous Injection of Foreign Protein	Fatal Injection: * Nature, Purpose, Amount	Interval Between Injection and Death	Clinical and Pathologic Findings
Sheppe [15]	♂ Negro 30 yr.	Probably given injections of antitoxin for war wound	Tetanus antitoxin Prophylaxis for recent bullet wound 1,500 units	20 min.	Symptoms in 10 min.; breathlessness; distended lungs
Ziskind and Schattenberg [14]	♀ Negro 39 yr.	0.06 cc. of typhoid vaccine 6 days ago	Typhoid vaccine Treatment of arthritis 0.15 cc. intravenously	30 min.	Collapse, sudden death, distended lungs

* Unless otherwise stated, the injection was subcutaneous.

TABLE 2.—*Deaths Following Injection of Foreign Protein: Asthmatic Cases*

Author	Sex, Race and Age	Previous Injection of Foreign Protein	Fatal Injection: * Nature, Purpose, Amount	Interval Between Injection and Death	Clinical and Pathologic Findings
Vance and Strassmann, case 6	♀ Negro 4 yr.	None	Intracutaneous injection of silkworm, etc. Test	3 hr.	Symptoms in 10 min.; shock, cyanosis, distended lungs
Lamson,[17] case 1	♂ White 14 yr.	None	Diphtheria antitoxin Prophylaxis 750 units in muscle	20 min.	Enlarged thymus at necropsy
London letter, J.A.M.A. 52: 223, 1909	♀ White 18 yr.	None	Diphtheria antitoxin Prophylaxis Dose (?)	10 min.	Asthmatic attack, distended lungs
Lund and Hunt [18]	♀ White 21 yr.	None	Guinea pig serum Experiment 0.2 cc. intracutaneously	14 min.	Asthmatic attack, distended lungs
Boughton [19]	♂ White 29 yr.	None	Horse serum for asthma 0.06 cc.	45 min.	Symptoms in 2 min.; asthmatic attack, distended lungs
Lamson [16]	♂ White 34 yr.	Injections of bermuda grass pollen extract	15th injection of pollen extract Treatment	5 min.	Asthmatic attack, distended lungs
Vance and Strassmann, case 7	♂ Negro 35 yr.	Injections of ragweed pollen extract	Injection of pollen extract Treatment	Less than 60 min.	Found dead; distended lungs
Gillette, H. F.: J.A.M.A. 50: 40, 1908	♂ White 52 yr.	None	Diphtheria antitoxin globulin For asthma 2,000 units under scapula	15 min.	Asthmatic attack, distended lungs

* Unless otherwise stated the injection was subcutaneous.

minutes. The symptoms were described as asthmatic in type, and the lungs were found to be inflated at necropsy.

Four patients lived several hours after injection of horse serum. The patient in our case 1 lived six hours with signs of severe dyspnea of the asthmatic type. In our case 3 the patient became comatose in

about thirty minutes and died after fourteen hours. The patient in Ferguson's [12] case presented difficult respirations and a rigid inflated chest about fifteen minutes after the injection and died two hours after the onset of these symptoms; the lungs were distended and filled with a serosanguineous exudate. The patient in the case reported by Gurd and Emrys-Roberts [6] had attacks of vomiting and a bloody diarrhea about an hour and a half after the injection, and later showed a rapid pulse, frequent respirations and a high temperature, with death occurring in about twenty-three hours; necropsy disclosed distended lungs, with fluid and red blood cells in the alveoli, and marked congestion of the liver and other viscera.

A microscopic examination was made in only 6 of the cases from the literature in table 1 and in 4 of our cases; as the findings in the latter have already been described, it is only necessary here to repeat the most important ones, namely, the distention of the alveoli of the lungs and cellular infiltration of the bronchial walls, which contained a varying number of eosinophilic leukocytes among other cells. In the case of Dean [13] the alveoli were normal in size and eosinophilic leukocytes were present in the bronchial wall and the sinusoids of the liver; the liver cells were described as vacuolated in appearance. The microscopic examination in the case of Ziskind and Schattenberg [14] disclosed distended alveoli and the presence of eosinophilic and neutrophilic leukocytes in the pulmonary capillaries and the sinusoids of the liver, but there was no description of a similar cellular infiltration of the bronchial wall. In the cases of Ferguson,[12] Bullowa and Jacobi,[8] Gurd and Emrys-Roberts [6] and Sheppe [15] mention was not made of any abnormal cellular infiltrate in the submucous layer of the bronchi, but marked distention of the pulmonary alveoli was described; there was, however, some fluid containing red blood cells in the alveolar and bronchial lumens in the cases of Ferguson [12] and Gurd and Emrys-Roberts.[6] The results of these examinations indicated that there was considerable difference microscopically among the various reported cases.

Table 2 lists 8 asthmatic cases, in which death occurred shortly after the injection of substances as follows: horse serum derivatives in 4 cases; pollen extract in 2 cases, a mixture of silkworm, sheep's wool and kapok (an intracutaneous test injection) in 1 case, and guinea pig serum in 1 case. The racial division of the patients was: white persons 6; Negroes 2. Five of the patients were males and 3 were females; the ages varied from 4 years to 52 years.

12. Ferguson, R.: Canad. M. A. J. **43**:469, 1940.
13. Dean, H. R.: J. Path. & Bact. **25**:305, 1922.
14. Ziskind, J., and Schattenberg, H. J.: Arch. Int. Med. **62**:813, 1938.
15. Sheppe, W. M.: J. Lab. & Clin. Med. **16**:372, 1930.

Six patients had not received any previous injections of protein but in our case 7 and in the case of Lamson [16] there had been regular injections of pollen extract for some time previously. The symptoms at the time of death were those of a typical asthmatic attack, and at necropsy the lungs were found to be inflated; however, Lamson [17] did not mention specifically the condition of the lungs in his case 1. The duration of the clinical course in this group varied from five to forty-five minutes.

Microscopic examination was reported in 4 cases: Our cases 6 and 7 and the cases of Lund and Hunt [18] and Boughton. [19] The principal features of our cases were the distention of the pulmonary alveoli and the marked cellular infiltration of the bronchial walls, especially by eosinophilic leukocytes. The microscopic observations in the case of Lund and Hunt [18] were similar. In the case of Boughton,[19] thickening in the bronchial wall and of the walls of the pulmonary arteries was described, the alveoli of the lungs were found to be distended and eosinophilic leukocytes were found in the spleen, but there was no mention made of the cellular infiltration in the bronchial wall.

COMMENT

Most of the cases in tables 1 and 2 presented either clinical signs or pathologic lesions in the lungs which indicated death by rapid or prolonged asphyxia resulting from spasm of the bronchial musculature. The typical clinical history was that of a sudden onset with difficult and even asthmatic respirations, starting immediately after the injection or from ten to thirty minutes later; death occurred within a few minutes or after a few hours from asphyxia. At necropsy the lungs were uniformly inflated with air, and occasionally the muscles of the bronchi were found to be contracted. There is no doubt that the patients had some sort of hypersensitiveness to the foreign protein in the injection fluid and that this was instrumental in bringing about the bronchial spasm. The condition has a striking resemblance to the experimental anaphylaxis in guinea pigs described by Auer and Lewis [20] and also to the fatal bronchial asthma observed in man in cases in which death occurred during a spontaneous asthmatic attack.

Two theories have been proposed to explain reactions to injections of foreign proteins in man which are characterized by asphyxia from bronchial spasm.

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16. Lamson, R. W.: J. A. M. A. **93**:1775, 1929.
17. Lamson, R. W.: J. A. M. A. **82**:1091, 1924.
18. Lund, H., and Hunt, E. L.: Arch. Path. **32**:664, 1941.
19. Boughton, T. H.: J. A. M. A. **73**:1912, 1919.
20. Auer, J., and Lewis, P. H.: J. Exper. Med. **12**:151, 1910.

The first theory has been applied particularly to explain the so-called true anaphylactic shock in man, which follows a previous injection of the protein in question. The hypersensitiveness thus induced is believed to be based on an antigen-antibody reaction in which the antigen is represented by the foreign protein, and the antibodies have been formed by the response of the reticuloendothelial cells of the body against the injected antigen. Some of the antibodies may circulate freely in the blood, while others attach themselves to certain structures, especially the smooth muscle fibers of the bronchi. After this junction has been effected, another injection of the specific antigen will remove the circulating antibodies by combining with them and then, if present in excess, will attach itself to the antigen already joined to the muscle fibers. If this second combination occurs in sufficient quantity, there may be a spasm of the bronchial muscles, with death by asphyxia.

Table 1 contains 6 cases in which the patient had been given horse serum or some other protein at varying intervals prior to the fatal injection. The cases of Koch,[7] Waugh,[21] Sheppe,[15] Dean,[13] Ziskind and Schattenberg[14] and our case 1 are possibly instances of anaphylaxis, but this would be difficult to prove positively.

The second theory has been proposed particularly to explain hypersensitivity due to a constitutional factor of a hereditary nature. In instances of this type the body is said to contain one or more specific sensitizing substances known as reagins which have a marked tendency to attach themselves to the tissue cells of the patient, usually the bronchial muscle fibers and cells of the reticuloendothelial system. While fixed in this fashion, the reagin may combine specifically with the corresponding antigen, known as allergen, and precipitate a bronchial spasm or some other type of allergic manifestation. Workers like Thommen[22] advocate the theory of an auxiliary factor termed the "tissue factor" which gives a plausible explanation for the variations in symptoms of two persons who possess the same reagin and are exposed to the same antigen. The tissue factor is believed to determine the clinical expression of the reaction to the allergen through certain organs known as "shock organs," which in one person may be the oculonasal tissues and in the other the bronchial musculature; as a consequence introduction of the specific antigen will produce symptoms of hay fever in the first and contraction of the bronchial musculature, causing an asthmatic attack in the second.

A good example of the hereditary nature of hypersensitiveness is furnished by the case of Lund and Hunt,[18] whose patient died after

21. Waugh, G. H.: Brit. J. Child. Dis. **15**:37, 1918.

22. Thommen, A. A.: Hay Fever, in Coca, A. F.; Walser, M., and Thommen, A. A.: Asthma and Hay Fever, Springfield, Ill., Charles C. Thomas, Publisher, 1931, p. 708.

an injection of guinea pig serum. Later it was discovered that the deceased person was asthmatic and that her family was subject to allergic complaints, most notably a cousin of her mother, whose death after an injection of diphtheria antitoxin was reported by Kortright.[11] As far as Lund and Hunt could ascertain, their patient had never had any previous contact with the specific allergen. However, they were able to transfer passively the reagin from her serum to local cutaneous areas in nonallergic subjects by intracutaneous injection; thereafter these areas became sensitive to guinea pig protein while the untreated skin remained insensitive.

Twelve patients whose cases are listed in table 1 did not give a history of a previous injection of foreign protein, though, of course, absorption of the antigen by other routes, such as those of inhalation or ingestion, could not be excluded. There was scarcely any difference, however, between the way in which the patients reacted to the fatal injection as compared with the reaction of the 6 with a history of having previously been given foreign protein; nor was there any essential difference between the reactions in the presumably nonasthmatic patients and the patients known to be asthmatic. Kellett [23] has suggested that after serum injections sudden deaths of the bronchial spasm type occur solely in patients known to be asthmatic or at least in those who are potentially asthmatic. This theory finds support in a number of facts: 1. Bronchial asthma, according to Macdonald,[2] is often latent, and some patients may not show symptoms until middle age. As pointed out by Vaughan [4] and Wiener and his collaborators,[24] many persons in the population have allergic inheritances from one or both parents, so that the degree of such an inheritance in the person will depend on the combinations of allergic genes which have been acquired from his parents. According to these workers, with bilateral allergic inheritance, the condition tends to manifest itself more regularly and earlier in life, while with unilateral allergic inheritance it tends to express itself later in life or may even remain latent. A certain number of these persons with lesser allergic inheritance may not be discovered until they receive injections of horse serum and signs of bronchial spasm develop. 2. The microscopic examinations in our cases, in the case of Lund and Hunt,[18] and in the case of Dean [13] show a similar type of cellular infiltration, especially of eosinophilic leukocytes, in the bronchial walls both in patients known to be asthmatic and in those who presumably were nonasthmatic. However, this type of infiltration was not reported in several cases listed in table 1. Because of this discrepancy the final decision as to whether or not the deaths following injection of serum in persons presumably nonasthmatic are examples of atopy can-

23. Kellett, C. E.: Practitioner **144**:276, 1940.
24. Wiener, A. S.; Zieve, I., and Fries, J. H.: Ann. Eugenics **7**:141, 1936.

not be made until more deaths of this sort are investigated macroscopically and microscopically at necropsies. It is the opinion of the authors that the 7 cases in our series and many cases reported in the literature really belong to the atopic group.

The influence of status lymphaticus on the production of a state of hypersensitiveness to injected foreign protein has been discussed since 1896, when the first deaths from this cause were reported. Symmers [25] has suggested that in early life, when the lymphoid tissues are hyperplastic, involutional changes in these structures may set a mechanism in motion which is represented in the tendency toward allergic reaction. This theory cannot be absolutely discounted as a possible explanation of the hypersensitiveness to foreign protein sometimes encountered in children and adolescents, but it will not explain the same type of hypersensitiveness in adults. In any event, the connection of status lymphaticus with anaphylactic or atopic conditions is not well defined and is not recognized by most observers as, for example, Lamson [26] and Bullowa and Jacobi.[8]

Certain investigators, like Dean [13] and Gurd and Emrys-Roberts,[6] have claimed on the basis of the symptoms and the lesions in their own cases that the anaphylactic reaction as described by Weil [27] in dogs is occasionally seen in man. In dogs there are massive engorgement of the liver and extensive congestion of the portal circulation, so that death supervenes from shock. Bauer and collaborators,[28] Vaughan [4] and Popper [29] declared that this reaction in dogs is referable to the smooth muscle bundles at the outlet of the hepatic veins, which contract and obliterate the lumens of these vessels, shutting off the outflow of blood from the liver and its portal tributaries. According to Popper,[29] man lacks such muscle bundles in his hepatic veins and probably the intensity of liver engorgement which has been described in dogs would not be possible in him. Kellett [23] also considered that anaphylaxis may be produced in some persons by contraction of the muscles of the pulmonary arteries, causing obstruction of the lesser circulation and failure of the right side of the heart, a condition similar to that described by Coca [30] as the anaphylactic reaction in rabbits. The contention that the reactions peculiar to rabbits and dogs are observed in human subjects is not susceptible of final proof or disproof at present, but at any rate is not supported by the available evidence.

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25. Symmers, D.: Am. J. Surg. **26**:7, 1934.
26. Lamson (footnotes 16 and 17).
27. Weil, R.: J. Immunol. **2**:525, 1917.
28. Bauer, W.; Dale, H. H.; Poulsson, L. T., and Richards, D. W.: J. Physiol. **74**:343, 1932.
29. Popper, H.: Klin. Wchnschr. **10**:2129, 1931.
30. Coca, A. F.: Arch. Path. **1**:96, 1926.

It is certain that the vast majority of anaphylactic reactions in man are manifested as a sudden spasm of the bronchial muscles which causes acute inflation of the alveoli of the lung and obstruction of the blood flow through the pulmonary capillaries, with passive congestion. This process will be more or less intense, depending largely on the sensitivity, the amount of serum injected and the route.

The antigen-antibody complex, or substances like histamine released by this combination, act on the endothelium of the capillaries and cause increased permeability of the vessel wall. This may explain the edema of the brain in our cases 2 and 3 and the edema of the laryngeal submucous layer in our cases 3 and 4. It also may explain the serosanguineous fluid in the lungs in the cases reported by Ferguson [12] and Gurd and Emrys-Roberts.[6]

It is easy to see how, with these factors in operation, clinical signs and symptoms which are similar to those observed in the anaphylactic reactions of dogs and rabbits are occasionally seen in human patients. They are, however, explainable on the basis of the disturbance in the circulation caused by the bronchial spasm and to a certain extent on the basis of the greater permeability of the capillary endothelium induced by the antigen-antibody complex or the substances released by it.

SUMMARY

The cases of 5 presumably nonasthmatic persons who died after injections of antitoxin and of 2 patients known to have bronchial asthma in whom death followed injections of foreign protein are reported here. All the patients showed marked inflation of the lungs and signs of asphyxia due to bronchial spasm. In cases 2 and 3 the brain was edematous and under compression. In cases 3 and 4 there was edema of the submucous layer of the upper portion of the larynx. Microscopic examination in 6 cases demonstrated varying numbers of eosinophilic leukocytes in the bronchial wall, suggesting that the reactions were hypersensitive in nature.

BILATERAL CORTICAL NECROSIS OF THE KIDNEY

A REPORT OF TWO CASES

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AND
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This report deals with 2 cases of symmetric cortical necrosis of the kidneys following toxic separation of the placenta.

This condition has received a fair amount of attention during the last ten years, as shown by the recent review by Duff and Murray.[1] According to these authors, the literature contains at present reports of 71 authenticated cases. In 48 of these cases the renal disease followed pregnancy. In the remaining 23 cases it was a complication of one or another type of infection or it followed absorption of "toxic" material. In the latter group 15 of the patients were males and 8 were females.

A number of cases are reported in the literature in which, though the clinical findings were those of symmetric cortical necrosis of the kidneys, the patients recovered after a varying period of anuria or extreme oliguria.

The first case was that of a white quintipara 33 years old. Her first two pregnancies were normal; the last two were complicated by preeclampsia. She entered the hospital seven weeks from term, with signs of toxic separation of the placenta and was delivered of a stillborn baby. The placenta showed gross evidence of premature separation. She was anuric on admission, and a total of only 30 cc. of urine was obtained during the next seven days. The nonprotein nitrogen of the blood rose gradually from 48 mg. to 115 mg. per hundred cubic centimeters. The blood pressure varied around 200 systolic and 100 diastolic. She had one attack of convulsions. She died on the seventh postpartum day.

The second case was that of a white quadripara 40 years old. About two months from term, she entered with signs of toxic separation of the placenta. Her previous pregnancies had been normal, and her past history was noncontributory. She was delivered of stillborn twins. The placentas showed the picture of premature separation. On admission only 12 cc. of urine was obtained. Post partum the total urinary output of eleven days was 53 cc. The nonprotein nitrogen of the blood rose from 42 mg. to 155 mg. per hundred cubic centimeters. On admission the blood pressure was 158 systolic and 120 diastolic; it rose to 210 systolic and 110 diastolic on the fourth day post partum, before an attack of convulsions, and ranged around 165 systolic and 80 diastolic toward the end. Death occurred on the eleventh postpartum day.

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1. Duff, G. L., and Murray, E. G. D.: Am. J. M. Sc. **201**:428, 1941.

In both cases at autopsy the kidneys showed symmetric cortical necrosis.

In the first case the kidneys weighed 285 Gm. each. They were swollen, and the capsules stripped easily, revealing smooth outer surfaces of a dark purplish hemorrhagic color. Against this background, multiple bright reddish spots, as well as small yellowish areas, stood out clearly. On the cut surfaces the cortex was of uniform width and well demarcated from the medulla. Multiple areas of necrosis were seen in the cortex, involving also the columns of Bertinin. The necrotic areas varied from bright red or hemorrhagic purple to yellow, but the hemorrhagic areas were more numerous and larger. A red zone surrounded the yellowish areas. There were some streaky, apparently uninvolved areas, and in some places a narrow zone of apparently intact cortex was noted immediately beneath the capsule. The pyramids presented well defined tubular striations. The calices and pelves and their mucosa were not grossly remarkable. The larger vessels at the hilus were found to be free from thrombi or appreciable sclerotic changes. The smaller vessels were not grossly remarkable.

Additional pertinent findings were small areas of necrosis in the liver, small ulcerations in the cecum and extensive subserosal hemorrhages in the uterus, mostly around the horns. Grossly, no remarkable changes were noted in the brain or the pituitary. Other significant findings were bronchopneumonia and cardiac hypertrophy (heart weight, 432 Gm.).

In the second case the right kidney weighed 214 Gm. and the left 200 Gm. Each was somewhat firm, and the thin capsule stripped easily, leaving a smooth outer surface. This surface was of blotchy appearance with small and large areas of a deep purplish red hemorrhagic color. The intervening tissue was of a yellowish tan color. On cut surfaces the parenchyma everted under the capsule. The cortex was of uniform width, appeared to be necrotic throughout and was of a yellowish color with reddish streaks and dots. In some instances the columns of Bertini were involved. The necrotic areas were sharply demarcated from the medulla by an irregular narrow reddish band. In some portions a narrow rim of apparently uninvolved cortex could be seen immediately beneath the capsule. The medulla appeared intact with well defined tubular striations. The calices and pelves and their mucosa were not grossly remarkable. The larger renal vessels were free from thrombosis or significant sclerotic changes, while the smaller branches were not grossly remarkable.

Further pertinent findings were multiple ulcers in the cecum and subserosal hemorrhages of the uterus, particularly around the horns. Of interest were some small petechial hemorrhages noted grossly in the pituitary near the stalk, in the stalk and in the tuber cinereum. Other significant findings were bronchopneumonia, cerebral and pulmonary edema, bilateral hydrothorax (500 cc. on each side) and ascites (400 cc.).

In both cases there was no evidence of infection. Cultures did not yield any growth.

MICROSCOPIC OBSERVATIONS

In the first case, the kidneys showed numerous, sometimes confluent, patchy areas of necrosis confined to the cortex.

The earliest lesions were in the walls of the afferent arterioles just before or at the point where the vessel enters the glomerulus and splits up into the capillaries forming the glomerular tuft. Here the media showed small eosinophilic patches of homogeneous appearance with loss of cellular detail (fig. 1*A*). The vessel itself at this point appeared rather dilated and congested. Deposits of conglutinated blood elements with or without fibrin over the degenerated areas were sometimes

present, but the vascular lumen appeared to remain partially patent, at least for some time. The glomerular endothelium and also the cells covering the glomerular tufts began to show swelling and early necrosis. Thrombosis appeared generally as a propagation extending distally from the site of the original lesion. Fibrinoid

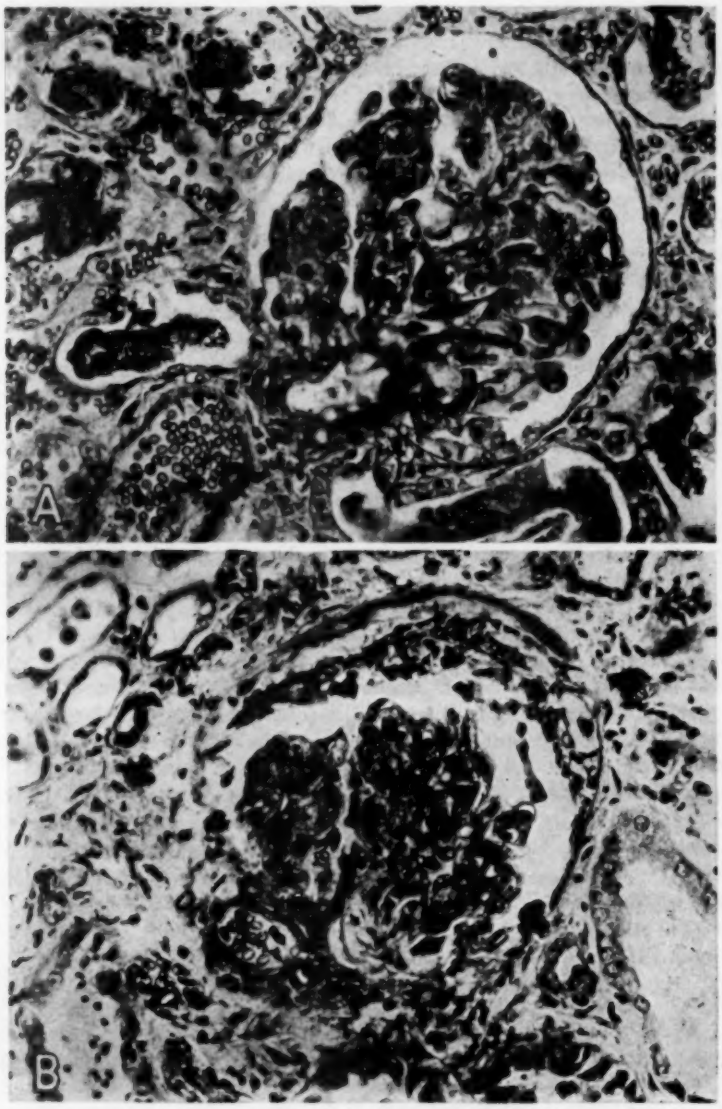

Fig. 1 (case 1).—Glomeruli (eosin–methylene blue; ×250): *A* shows an early vascular lesion in the afferent arteriole. *B* shows repair with proliferation of capsular epithelium and formation of adhesions between the glomerular tuft and capsule. (This is interpreted as an arrested stage of the early vascular lesion followed by repair.)

degeneration of the arteriolar wall was associated with hemorrhage into the wall and surrounding tissue. Sometimes complete obstruction of the arteriolar lumen by thrombi resulted. The thrombi were not of a laminated appearance but consisted of conglutinated degenerating blood elements and mostly, although not always, of fibrin. Hemorrhage into the glomerulus was present as a result of these changes.

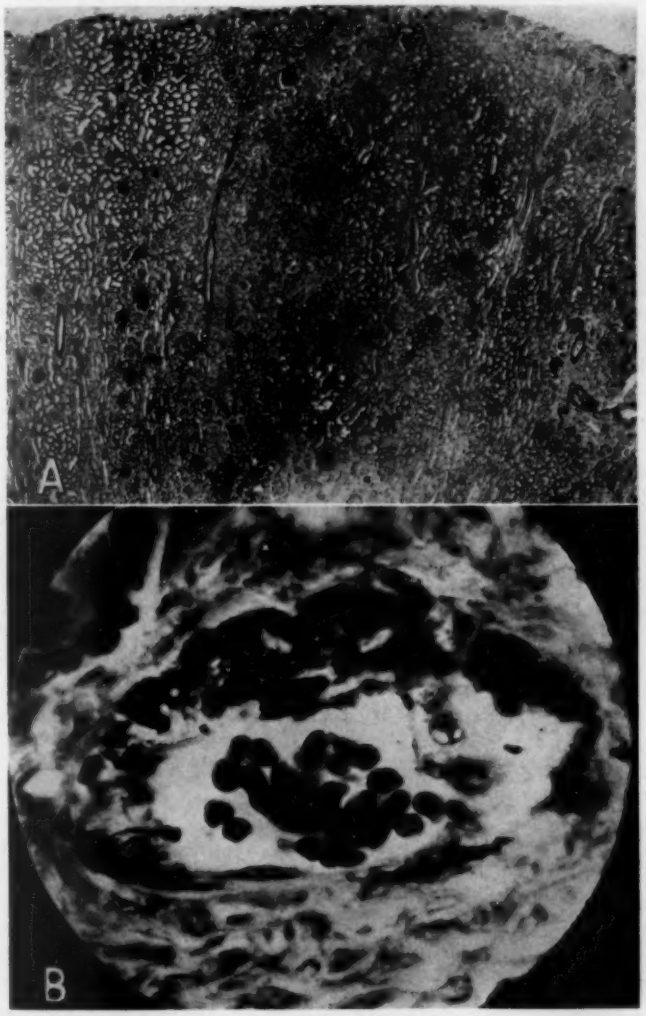

Fig. 2.—*A*, lesion in renal cortex in case 1. Note the patchy or focal nature of the lesion. Necrosis of varying age is seen on the right; uninvolved cortex, on the left. Hematoxylin and eosin; × 17. *B*, arteriole in a renal capsule in case 2, showing varying stages of degeneration in the media. Eosin–methylene blue; × 750.

In a few instances the process appeared to be arrested before complete obstruction of the afferent arteriole occurred. In such cases the degenerative processes in the glomerular endothelium and covering cells became more marked and

involved also the lining cells of Bowman's capsule. Proliferation of these cells with crescent formation and adhesions between the tufts and the capsule resulted (fig. 1 *B*).

Generally the degenerative process in the wall of the afferent arteriole appeared to continue by retrograde extension. Frequently it involved the intralobular arteries and occasionally reached the interlobar arteries. The same sequence of events as described in the arterioles, with final thrombosis of the vessel, might take place. There was no evidence of organization of these thrombi. In the older lesions, large monocytes with foamy fat-containing cytoplasm appeared in the adventitia of the vessels.

Following the vascular lesions, patchy areas of ischemic necrosis developed in the cortex (fig. 2 *A*). The tubular lesions began in the proximal convoluted tubules. The capillaries between the tubules were congested but free from thrombi. There were a moderate degree of polymorphonuclear leukocytic infiltration and some repair of the tubular epithelium.

In the uninvolved areas of the cortex the glomeruli, the afferent arterioles and the intralobular arteries appeared contracted and were practically empty. They showed some sclerotic changes. The tubular epithelium was well preserved. The capsule over the narrow rim of uninvolved cortex immediately beneath it showed only some dilated capillaries.

The medulla and the interstitial connective tissue, as well as the pelvic epithelium and connective tissue, were not remarkable.

In the second case the findings in the kidneys were essentially the same, although more extensive and advanced. The same sequence of events leading from the earliest lesion in the wall of the afferent arteriole to extensive areas of ischemic necrosis was seen, thus indicating that the process was still active. The intralobular and interlobar arteries displayed more frequent and more extensive necrosis of their walls. None of the thrombi showed organization.

Polymorphonuclear leukocytic infiltration had increased, and repair was more marked. Large fat-laden monocytes in the walls of the necrotic arteries were conspicuous. Free fat in small droplets was seen in the lumens of the arteries and tubules in the necrotic areas. Some few areas untouched by necrosis were present. As in the first case, the glomerular capillaries, the afferent arterioles and the next larger arterial branches appeared contracted and contained little or no blood. They did not show sclerotic changes. The medulla, the pelvic epithelium and the connective tissue were not remarkable.

Of interest were the capsule and the adjoining areolar fat tissue in relation to the narrow subcapsular areas of cortex which had escaped necrosis. Here a few of the arterioles showed small patches of early degeneration of their media similar to that described (fig. 2 *B*).

The same degenerative changes of the arteriolar walls were found in the ulcerative lesions of the cecum in both cases. Since the lesions were in an early stage, the sequence of events could be followed easily.

Lesions of the adrenal were present only in the second case. They were of a very early type, involved only small areas and consisted of the same degenerative changes of the walls of the arterioles in the cortex just beneath the capsule. Thrombi had begun to form and extend into the sinusoids of the cortex but they did not appear to obstruct the sinusoidal lumens completely. The adjoining cells of the zona fasciculata showed beginning degeneration.

Hepatic lesions were present only in the second case and consisted of some centrolobular or midzonal necrosis of the usual type.

As one would expect, the uterine lesions in these 2 cases yielded little information, since both patients were several days post partum.

In the first case, only the pituitary and a good portion of its stalk were available for microscopic study. Here the lesion was confined to the anterior lobe of the organ, while the remaining portions were entirely uninvolved. The lesion consisted of a large area of ischemic necrosis occupying the central portion of the anterior lobe (fig. 3*A*). Some thrombi were found in the sinusoids of the

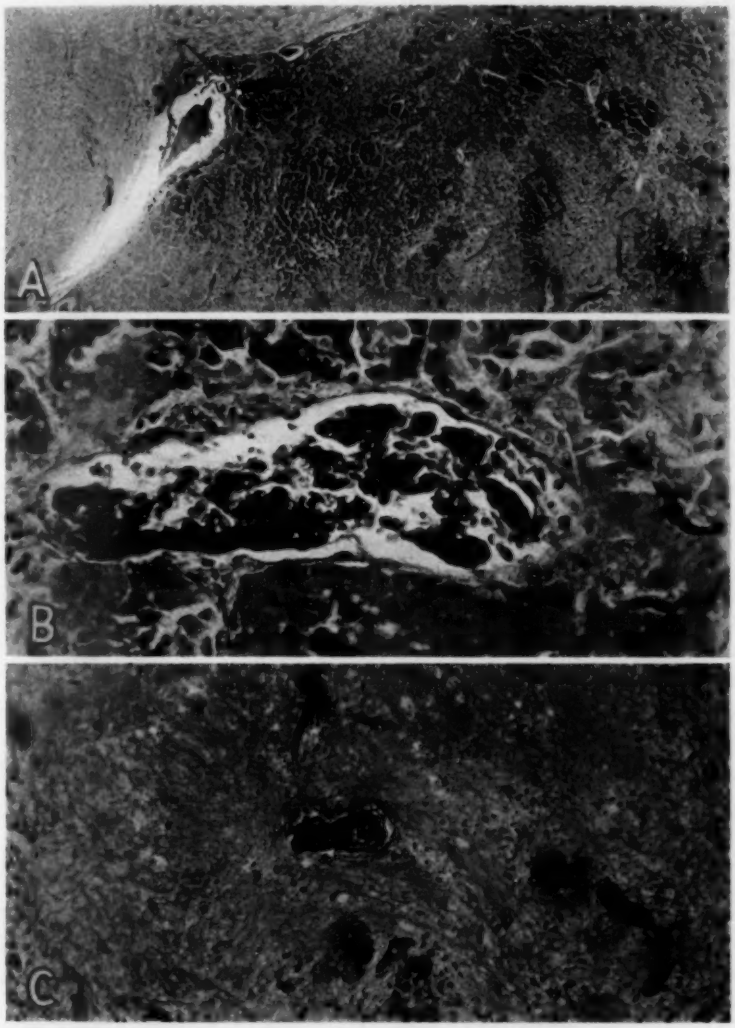

Fig. 3.—*A*, pituitary with portion of its stalk, showing an area of ischemic necrosis in the anterior lobe (case 1). The enclosed area is seen in *B*. Eosin–methylene blue; × 17. *B*, thrombus with organization in a sinusoid of the anterior lobe of the pituitary. Eosin–methylene blue; × 250. *C*, area of necrosis around arterioles which are the seat of medial degeneration and thrombosis (from the floor of the third ventricle, in the region of the tuber cinereum, in case 2). Hematoxylin and eosin; × 115.

necrotic area, a few of which showed beginning organization (fig. 3*B*). In the intact portions of the anterior lobe the cells were of the usual type and distribution if one makes allowance for the presence of the so-called pregnancy cells.

In the second case the pituitary and its entire stalk, as well as the region from the floor of the third ventricle, were available for microscopic study. Here a necrotic area quite similar to that described in the first case was present in the anterior lobe of the pituitary, although no thrombi were found in the sinusoids. A few small arterioles in the capsule of the inferior surface showed some degeneration of their walls and contained thrombi. The intact areas of the anterior lobe were not unusual except for the still increased number of the so-called pregnancy cells. The pars intermedia and the pars tuberalis, as well as the posterior lobe, were not remarkable.

The degree of basophil infiltration of the posterior lobe was not unusual in either case.

The lesions of the pituitary stalk consisted of occasional thrombosed sinusoids and a few small areas of early necrosis in the surrounding tissue. There was one arteriole showing degeneration of its wall. A sinusoid coming from this arteriole was dilated but not otherwise involved.

Similar but more extensive patchy lesions of varying age were seen in the tuber cinereum. Again the primary lesion involved the arteriolar wall. There were a number of small, sometimes confluent areas of ischemic necrosis around these damaged vessels (fig. 3*C*). Some of the necrotic areas were quite old, with evidence of repair, while others were of short duration, with recent hemorrhage. The larger arteries in the leptomeninges covering the tuber cinereum were not remarkable. These lesions were confined to the tuber cinereum and were not found elsewhere around the third ventricle or in the other routine sections of the brain.

In both cases, sections from all other organs were examined but failed to show the described vascular lesions. Some arteriolosclerosis was present in the first case, chiefly seen in the spleen, pancreas and adrenal capsule.

COMMENT

The cause of symmetric necrosis is unknown. At the beginning this condition was believed to occur in pregnancy only. But the number of cases in which it was unassociated with pregnancy has increased steadily.

As to the genesis of the condition, varying opinions have been advanced, which have been reviewed by Duff and Murray.[1] Their conclusions are that some unknown factors, given suitable conditions, cause disturbances in the terminal arteries of the renal cortax. The damage done by these factors depends on the sensitivity of these vessels to irritation. Experiments indicate that these vessels, which even normally are particularly receptive to stimulation, may become hypersensitive under certain conditions, such as pregnancy. Variations in the degree of hypersensitivity of these vessels and in the intensity of the irritating factor may cause a series of vascular disturbances ending in partial or complete necrosis of the arterial wall. Vascular spasm or the direct action of some toxic substance may be responsible for this necrosis. Thrombosis

may take place at any time during the process. This hypersensitivity of the renal arteries is present, although less marked, in the same-sized arteries of other organs.

Lesions such as those found in the anterior lobe of the pituitary in both our cases and in the pituitary stalk and the tuber cinereum in our second case have not been recorded in the literature.

Only in three reports in the literature are cerebral lesions mentioned. They are described as petechial hemorrhages in the pons, midbrain, basal ganglions and choroid plexus.[2]

When one evaluates the lesions present in the various organs in our cases, it becomes evident that the process was everywhere active and still in progress. The extensive changes in the kidneys as well as those in the pituitary and the tuber cinereum were all of some standing and of about the same age. Besides these older and still progressing lesions, identical recent lesions were present in the cecum and the adrenal.

All lesions, regardless of age or location, appeared to form in a similar manner: There was degeneration of the arteriolar wall near or at the point of division of the arteriole into capillaries. In most instances the degeneration led to gradual obstruction of the vessel lumen by thrombi. Hemorrhage into the arteriolar wall and surrounding tissue followed, with resulting ischemic necrosis. Thrombosis, however, did not appear to be a necessary factor, since ischemic necrosis of the tissue sometimes directly followed the lesion in the arteriolar wall. Retrograde extension of the degeneration in the arteriolar wall with or without accompanying thrombosis occurred in most instances. With the involvement of more and more arterioles and subsequent retrograde extension, ischemic necrosis involved continually larger areas. The extent of this necrosis varied with the presence of collateral circulation in the different organs.

In each kidney there were a few areas where the degenerative process in the afferent arteriole did not lead to total obstruction of the blood flow. In such areas the degenerative changes in the corresponding glomerulus developed further and were followed by repair. In this manner rare glomeruli displayed an appearance resembling that of chronic glomerulonephritis (fig. 1 *B*). This finding is of interest in view of those cases in which the patient recovered after having shown the clinical symptoms of symmetric cortical necrosis of the kidney. The lesions occurring during the period of extreme oliguria or anuria might, if extensive enough, lead to renal failure at a later stage. The original character of the renal lesions then may be obscured beyond recognition by superimposed secondary changes.

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2. Larson, C. P., and Bennett, R. J.: West. J. Surg. **47**:481, 1939. Jardine, R., and Kennedy, A. M.: Lancet **2**:116, 1920. Weaver, R. G., and von Haam, E.: Arch. Int. Med. **63**:1048, 1939.

The character of the lesions which involved the arterioles, their presence in the kidneys at a point where the juxtaglomerular apparatus of Goormaghtigh is situated, and the involvement of the anterior lobe of the pituitary, its stalk and the tuber cinereum must invite speculation. This speculation might tend to consider some sort of vasomotor disturbance, perhaps due to or associated with an alteration of the equilibrium of interrelated hormonal functions. Such reasoning, however, remains at present pure speculation, unsupported by sufficient evidence, experimental or otherwise.

SUMMARY

Two cases of bilateral cortical necrosis of the kidney following toxic separation of the placenta are presented. The renal lesions consisted of degeneration in the wall of the afferent arteriole at the point where the vessel enters the glomerulus. This degeneration was in most instances associated with thrombosis of the vessel. Retrograde extension of the process in the intralobular and interlobar arteries followed and led to ischemic necrosis of the kidney. Similar vascular lesions resulting in necrosis were found in the anterior lobe of the pituitary, in the pituitary stalk and in the region of the tuber cinereum at the floor of the third ventricle. They were also seen in the cecum and the adrenal.

RELATIONSHIP OF HEART SIZE TO CHOLESTEROL CONTENT IN EXPERIMENTAL ATHEROMATOSIS OF THE RABBIT

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It has long been a controversial issue whether or not myocardial ischemia due to narrowing of the coronary artery per se may be the cause of cardiac hypertrophy. Many observers [1] have expressed the opinion that the enlargement of the heart seen in association with severe coronary sclerosis and myocardial fibrosis is always the result of concomitant hypertension or valvular disease. On the other hand, patients who have been followed for years and have shown no hypertension at any time during their clinical course have been found at necropsy to have markedly enlarged hearts in the absence of any anatomic abnormalities other than coronary sclerosis and myocardial fibrosis.[2] A recent study [3] would indicate that when both hypertension and coronary sclerosis are present, each contributes to the ultimate cardiac enlargement.

In an attempt to determine what role, if any, myocardial ischemia plays in cardiac hypertrophy, a study was undertaken in which coronary atherosclerosis with all its sequelae was produced in rabbits by the feeding of diets high in cholesterol.[4] The presence of hypertension in these animals was excluded by direct measurement of the blood pressure, arterial puncture being used, and dynamically significant aortic valvular lesions, by photographic representation of the pulse contour. The results showed conclusively that the hearts of these animals were definitely enlarged, and this was attributed, therefore, to the coronary sclerosis

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This study was aided by grants from the A. D. Nast Fund for Cardiac Research and the Nelson Morris Fund.

1. Bell, E. T., and Clawson, B. J.: Arch. Path. **5**:939, 1928. Nathanson, M. H.: Am. J. M. Sc. **170**:240, 1925.

2. Davis, D., and Blumgart, H. L.: Ann. Int. Med. **11**:1024, 1937. Shohet, A. S.; Taub, S. J., and Kupersmith, H.: Illinois M. J. **77**:240, 1940.

3. Kahn, J. R., and Ingraham, E. S.: Arch. Path. **31**:373, 1941.

4. Katz, L. N.; Sanders, A.; Megibow, R. S., and Carlen, S.: Am. J. M. Sc. **200**:731, 1940.

and resultant ischemia. In reviewing this study, it occurred to us that the increase in heart weight might be accounted for, at least in part, by an increase in cholesterol deposits within the heart itself. Previous studies of quantitative cholesterol determinations [5] had shown that the cholesterol content of organs of the body is increased by high cholesterol feeding. With this in mind, we subjected rabbits to the identical treatment previously employed, and the hearts of these animals were analyzed quantitatively for cholesterol, the results being then compared with those obtained in an untreated, control group.

METHOD

A group of 14 rabbits, all less than 6 months of age, were fed a high cholesterol diet, and a group of 10 rabbits of identical age and size were maintained on a stock diet of alfalfa, oats and green vegetables. The high cholesterol diet was prepared by dissolving 25 Gm. of cholesterol in 500 cc. of cottonseed oil and adding 110 cc. of this mixture to each 500 cc. of cracked wheat used. The vitamin B content of this mixture was enhanced by the addition of a vitamin B concentrate in liquid form. Parke, Davis & Company supplied this material. In an attempt to minimize the ill effects of this poorly balanced diet, the rabbits were also fed fresh green vegetables twice weekly.

The animals were kept on this diet for periods varying up to four months, at the end of which time those that survived were killed. None of the rabbits in this group succumbed within the first two months of the diet, but only 4 lived for the full four month period. Those animals surviving for the longest periods appeared definitely undernourished and apathetic. All 14 were subjected to careful postmortem examination. Since the hearts were utilized for chemical analyses, microscopic examinations were not possible on all these organs; only the hearts of the last 2 rabbits were used for microscopic examination, and thus they are not included in the tables.

The 12 hearts used for chemical analyses were removed from the body with 1 mm. of aorta and pulmonary artery, and the wet weights obtained. These hearts were then macerated and dried to constant weight in an incubator at 100 C. for a period of eighteen hours. The residue was extracted for cholesterol by four repeated washings and gentle boilings in four 25 cc. samples of acetone-alcohol solution (consisting of equal parts of acetone and absolute alcohol). The mixture was then filtered and the filtrate subjected to analysis for cholesterol by the method of Schoenheimer and Sperry [6] modified for the photoelectric colorimeter. Dr. I. Kaplan, of the department of chemistry, suggested the use of this chemical procedure.

OBSERVATIONS AT AUTOPSY

The gross postmortem findings in all of the animals were quite consistent. The liver was definitely pale and slightly greasy to touch,

5. Aylward, F., and Stott, W.: Biochem. J. **31**:2055, 1937. Eberhard, T. P.: Arch. Path. **21**:616, 1936. Page, I. H., and Menshick, W. J.: J. Biol. Chem. **97**:359, 1932. Weinhouse, S., and Hirsch, E. F.: Arch. Path. **30**:856, 1940.

6. Schoenheimer, R., and Sperry, W. M.: J. Biol. Chem. **106**:345, 1934.

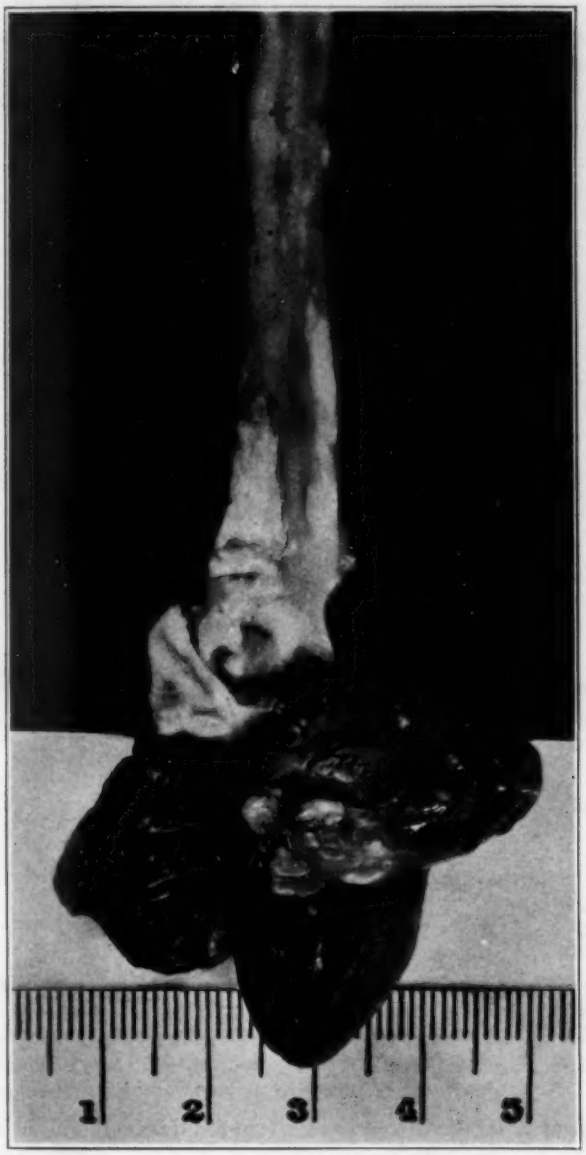

Fig. 1.—Heart and aorta of experimental rabbit 11 (table 2), showing extensive intimal atheromatosis of the ascending aorta and of the arch. Note the small atheromatous patches above the openings of the intercostal vessels (scale in cm.).

giving a distinct gross impression of fatty metamorphosis. The adrenal glands were somewhat paler than normal but otherwise presented no grossly visible abnormalities. The spleen, the kidneys, the liver and the gastrointestinal tract appeared normal. The lungs were normal save in those animals that died of intercurrent pneumonia. The pericardial cavity showed no noteworthy changes. The heart appeared grossly

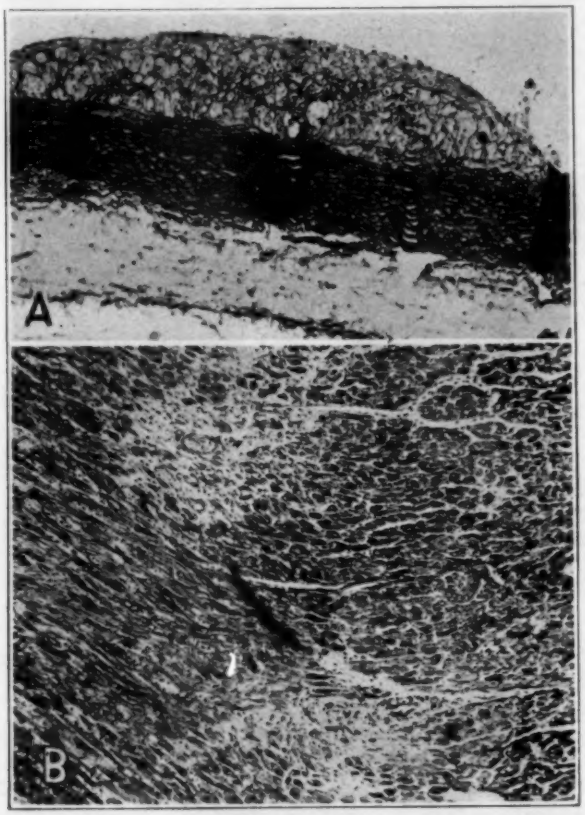

Fig. 2.—*A*, low power view of aorta (hematoxylin-eosin; × 56), showing proliferation and hyperplasia of the intimal cells, which are foamy in appearance. The remainder of the section appears normal. *B*, low power view of a section of myocardium (hematoxylin-eosin; × 40), showing scattered areas of fibrosis.

enlarged in almost all of the cholesterol-fed rabbits, and this enlargement appeared to be diffuse, involving all chambers. The aorta showed intimal atheromatous patches (fig. 1) in all but 1 of the cholesterol-fed rabbits, and in about half of them the pulmonary arteries disclosed similar lesions. In 2 instances, calcification was encountered in the atheromatous patches, but in none of these was ulceration seen. In

several instances, small atheromatous plaques were seen in the aortic leaflet of the mitral valve. There were no grossly visible myocardial lesions. Gross examination of the coronary arteries was difficult because of their minute size, but in several instances small areas of intimal atheroma could be seen. In only 1 rabbit of the control series could any atheromatous lesion be found, and this will be discussed subsequently.

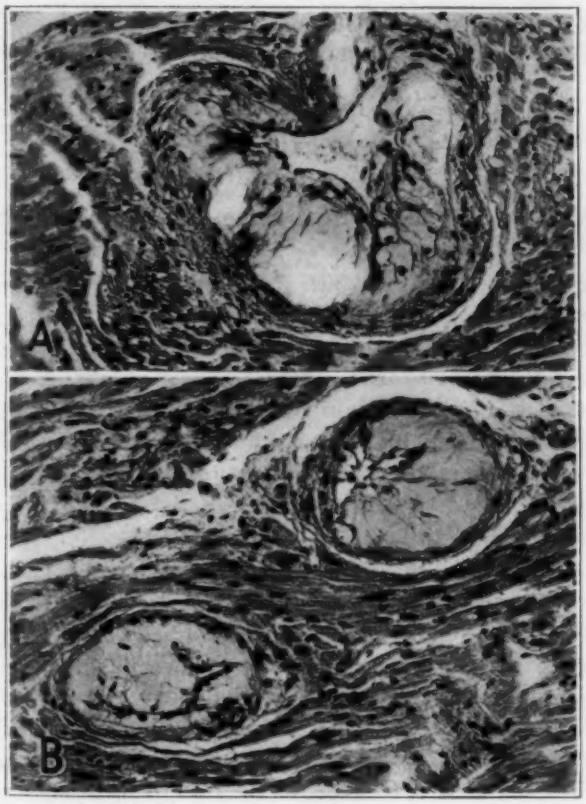

Fig. 3.—*A*, high power view of a section of myocardium (hematoxylin-eosin; × 128), showing involvement of one of the smaller coronary arteries. *B*, section showing extreme involvement of very small branches of the coronary arteries with almost complete occlusion of their lumens by the intimal lesions (hematoxylin-eosin; ×160).

Microscopic sections of the myocardium, coronary arteries and aorta were available from 2 of the cholesterol-fed animals.

The lesions in the aorta were confined entirely to the intima, the intimal cells showing proliferation and hyperplasia with marked increase

of cytoplasm, which was foamy in appearance, giving the impression that the cells had taken up fat (fig. 2 *A*). Most of the large branches of the coronary arteries showed lesions identical with these. The small branches of the coronary arteries showed extreme involvement in many sections (fig. 3), although some of them appeared to have been spared. Here, also, the changes were intimal, and marked hyalinization was apparent. The lumens of the involved vessels were markedly decreased, some retaining no more than a tenth of their original diameters. Small scattered areas of myocardial fibrosis were encountered (fig. 2 *B*), but there was no evidence of myocardial infarction, either recent or old.

These observations were thus identical with those previously reported from this laboratory.[4]

HEART WEIGHTS

The body weights and heart weights of both the control and the cholesterol-fed series are summarized in tables 1 and 2.

To eliminate any errors which might result from variations in the moisture content of the hearts, only the dried heart weight was used in the correlation with the body weight. Moreover, the heart weight of each rabbit was compared with the body weight of that animal, rather than with an average normal; this removed any source of error which might arise from undernourishment. The weights of the dried hearts of the cholesterol-fed animals ranged between 0.563 and 1.260 Gm., averaging 0.952 Gm., while those of the control rabbits ranged between 0.336 and 0.946 Gm., averaging 0.64 Gm.

Table 3 illustrates the percentage of the total body weight that the heart weight represents in each series of animals. It will be readily seen that the heart weights of the cholesterol-fed rabbits represent definitely larger percentages of the total body weights than do those of the control group. The percentages of the cholesterol-fed rabbits range between 0.044 and 0.10, with an average of 0.07, while those of the control series range between 0.022 and 0.054 with an average of 0.04. In 1 control rabbit, the percentage of the total body weight represented by the heart weight fell into the range of the cholesterol-fed group, and it is noteworthy that this was the only animal in the control group which showed spontaneous atheromatosis. The relative increase in the weight of this rabbit's heart appeared to be associated with the atheromatosis, as the cholesterol content of the heart was not increased. In 1 animal of the cholesterol-fed series, the percentage of total body weight represented by the heart weight fell within the range of the control normal group, and it is interesting to note that this was the only rabbit on the high cholesterol diet which failed to show any evidence of atheroma after a prolonged period on the experimental diet.

TABLE 1.—*Cholesterol Determinations on Hearts of Control Rabbits*

Rabbit	Weight of Rabbit, Gm.	Weight of Heart, Gm.	Weight of Dried Heart, Gm.	Total Cholesterol, Mg. per Gm. of Heart	Free Cholesterol, Mg. per Gm. of Heart	Cholesterol Ester, Mg. per Gm. of Heart	Cholesterol in Form of Ester, %
1	1,818	2.74	0.500	3.24	2.63	0.61	16
2	909	2.04	0.417	2.82	2.36	0.46	18.8
3	1,818	3.57	0.821	1.89	1.14	0.75	39
4	1,453	3.30	0.557	2.51	1.81	0.70	28
5	1,568	3.03	0.720	3.82	2.09	1.73	45.3
6	1,910	3.90	0.808	2.48	1.70	0.78	31.5
7	700	2.0	0.336	1.86	1.77	0.09	4.9
8	1,620	3.8	0.665	1.65	1.22	0.43	26
9	1,636	3.3	0.603	1.98	1.42	0.56	28.2
10*	1,740	4.67	0.946	2.46	1.61	0.85	34.5
Average.....		3.2	0.64	2.47	1.78	0.69	31.2

* This rabbit showed early spontaneous atheromatosis.

TABLE 2.—*Cholesterol Determinations on Hearts of Cholesterol-Fed Rabbits*

Rabbit	Days on Diet	Weight of Rabbit, Gm.	Weight of Heart, Gm.	Weight of Dried Heart, Gm.	Total Cholesterol, Mg. per Gm. of Heart	Free Cholesterol, Mg. per Gm. of Heart	Cholesterol Ester, Mg. per Gm. of Heart	Cholesterol in Form of Ester, %
1	80	1,363	3.36	1.467	2.11	0.93	1.18	56.0
2	83	1,150	3.20	0.563	11.40	8.00	3.40	29.5
3	95	1,085	3.85	0.706	14.40	9.60	4.80	33.3
4	108	1,310	4.33	0.787	8.82	2.25	6.57	74.5
5	126	1,370	3.27	0.730	7.20	2.15	5.05	70.1
6	126	1,370	3.94	0.950	10.08	2.55	7.53	74.7
7	131	1,246	3.44	0.968	7.36	2.53	4.83	65.6
8	131	1,200	3.80	0.767	10.01	4.25	5.76	57.5
9	72	1,900	5.45	1.510	10.59	4.87	5.72	54.0
10	79	1,285	2.77*	0.568	6.16	3.60	2.56	41.5
11	85	1,253	4.73	1.168	13.18	4.42	8.76	66.4
12	89	1,595	6.54	1.260	9.89	2.86	7.03	71.0
Average.....			4.07	0.952	9.27	3.83	5.27	57.8

* This rabbit showed no atheromatosis.

TABLE 3.—*Dried Heart Weight Expressed as Percentage of Total Body Weight of Animal*

Normal Rabbits	Cholesterol-Fed Rabbits
0.029	0.100
0.045	0.048
0.045	0.068
0.031	0.060
0.045	0.053
0.041	0.070
0.048	0.075
0.041	0.064
0.036	0.079
0.054 *	0.044 †
	0.093
	0.060
Average 0.041	Average 0.070

* This rabbit had spontaneous atheroma.
† This rabbit had no atheroma.

CHOLESTEROL ANALYSIS

The total cholesterol content of each heart was calculated in terms of milligrams of cholesterol per gram of dried heart (tables 1 and 2). The hearts of the cholesterol-fed series had total cholesterol contents varying between 2.11 and 13.18 mg., with an average of 9.27 mg., per gram of dried heart. Those of the control series showed total cholesterol contents ranging between 1.65 to 3.24 mg., with an average of 2.47 mg., per gram. of dried heart. Analyses for the amounts of free cholesterol, as well as the percentages existing in the esterized form, were carried out. In the cholesterol-fed series, the free cholesterol contents ranged between 0.93 and 9.60 mg. per gram. of dried heart, with an average of 3.83 mg. per gram., while the control series ranged between 1.14 and 2.63 mg. per gram of dried heart, with an average of 1.78 mg. per gram. The percentage of cholesterol esters in the cholesterol-fed series varied between 29.5 and 74.7, with an average of 57.8, while in the control series it ranged between 5.0 and 45.3, with an average of 31.2.

It can be seen that the increase in cholesterol content of the heart, although definite, accounts for less than one twenty-fifth of the increase in the weight of the heart. It is obvious that an increase of this magnitude could in no way account for the weight increase described in the foregoing section.

SUMMARY

Anatomically demonstrable sclerosis of the coronary arteries with resultant myocardial fibrosis was produced in rabbits by the feeding of diets high in cholesterol. A comparison of the heart weights of these rabbits with those of a control series showed, as reported previously,[4] that there is a distinct increase in the size and weight of the heart in the cholesterol-fed group and that this increase is reflected in the percentage of the total body weight comprised by the heart weight.

The hearts of the cholesterol-fed group were analyzed quantitatively for cholesterol and the results compared with those obtained in a control series. It was found that the cholesterol-fed animals showed a definite increase in the cholesterol content of the heart both in the free and in the esterized form. This increase, though unequivocal, could in no way account for the increased weight of the heart.

Since previous studies [4] exclude the dynamic significance of lesions of the aortic valve, and since aortic sclerosis per se does not cause an increase in the work of the left ventricle,[7] we feel justified in concluding that myocardial ischemia resulting from atherosclerosis of the coronary arteries per se can be the sole cause of cardiac hypertrophy.

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7. Fahr, G.; Davis, J.; Kerkhof, A.; Hallock, P., and Giere, E.: Am. J. Physiol. **101**:376, 1932.

EXPERIMENTAL STUDIES IN CARDIOVASCULAR PATHOLOGY

VI. PECTIN ATHEROMATOSIS AND THESAUROSIS IN RABBITS AND IN DOGS

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Previous investigations had shown that a causal relationship exists between macromolecular colloidal disturbances of the blood plasma and the development of atheromatous and sclerotic lesions of the arteries (Hueper[1]). The evidence advanced indicated that the mechanism operative in the production of these vascular changes consists in the formation on the intima by the pathogenic colloids of films or precipitates which interfere with the proper oxygenation and nutrition of the walls of the vessels. It was demonstrated that proteinic colloids (gelatin, azoproteins) give rise to sclerosing lesions, while lipoidal and carbohydratic, emulsion-forming colloids produce vascular atheromatosis in addition to storage phenomena in various organs (cholesterol, polyvinyl alcohol, methyl cellulose, acacia).

For further confirmation and elaboration of this general principle, another macromolecular substance, pectin, was studied. Colloidal solutions of this macromolecular carbohydrate were injected intravenously into rabbits and dogs, and the effects of this treatment on the blood and on the internal organs were determined. The following report presents the results of these experiments.

EXPERIMENTAL OBSERVATIONS

Pectinum (pure citrus pectin of an average molecular weight of 200,000), which was obtained through the courtesy of Dr. G. Joseph, of the California Fruit Growers Exchange, was used. Citrus pectin belongs to the group of carbohydrate-like macromolecular substances possessing a long-chained molecule which consists of polymerized galacturonic acid molecules with various additional groups, such as galactose, araban, acetic acid and methyl alcohol. The purified product employed in these studies is a pale greenish yellow fine powder that dissolves readily in water when wetted previously with 95 per cent alcohol, giving a clear, slightly yellowish tinted viscous colloidal solution with an acid reaction

From the Warner Institute for Therapeutic Research.

1. Hueper, W. C.: Arch. Path. **28**:510, 1939; **31**:11, 1941; Pharm. Arch. **12**: 49, 1941; Medicine **20**:397, 1941; Arch. Path. **33**:1 and 267, 1942; Am. J. Path. **18**: 895, 1942.

(1.2 per cent solution—p_H 3.2). This solution, which is an excellent emulsifying agent for gases or liquids, gels on the addition of alcohol but is not stable when subjected to prolonged heating or when brought to a neutral or an alkaline reaction. A brownish precipitate is formed under such conditions, and the solution loses greatly in viscosity because of progressive depolymerization of the pectin molecule (Dehn [2]; Myers and Baker [3]; Hinton [4]; Joseph [5]). There exists a linear relation between the degree of viscosity and the concentration of pectin, as a 1 per cent solution has at 19 to 20 C. a viscosity of 16.08, a 0.5 per cent solution one of 8.44, a 0.25 per cent solution one of 3.22 and a 0.15 per cent solution one of 2.0 to 2.1, or about that of normal plasma. The osmotic pressure of a freshly prepared 1 per cent solution of this pectin is 84 cm. when tested against water.

For the purpose of the experiment, the p_H of the pectin solution was adjusted to about the neutral point (p_H 6.5 to 7.0) either by the addition of lime water

TABLE 1.—*Hematic Reactions in Dog 797, Weighing 6.3 Kg., After a Single Intravenous Administration of 20 cc. of a 1 per Cent Pectin Solution*

	Erythrocytes per Cu. Mm.	Sedimentation Rate, Mm.	Coagulation Time, Min.	Leukocytes per Cu. Mm.	Polymorphonuclears, per Cent	Lymphocytes, per Cent	Viscosity	
							Whole Blood	Plasma
Start	6,230,000	2.0	10½	13,000	90	8	7.36 @ 18.3 C.	2.09 @ 22.5 C.
5 minutes	6,920,000	39.0	6½	1,800	58	40	7.26 @ 19.0 C.	2.40 @ 22.6 C.
15 minutes	7,280,000	41.0	2½	3,000	92	7	7.40 @ 19.2 C.	2.25 @ 22.7 C.
45 minutes	6,360,000	46.0	6	3,600	94	6	6.04 @ 19.8 C.	2.23 @ 22.7 C.
2 hours	6,140,000	42.0	7	5,800	96	2	6.04 @ 20.0 C.	2.19 @ 22.8 C.
4 hours	5,890,000	44.0	6	9,200	98	2	6.38 @ 20.2 C.	2.15 @ 22.8 C.
6 hours	6,180,000	16.0	7	10,200	98	2	5.86 @ 20.4 C.	2.13 @ 22.8 C.
8 hours	5,610,000	2.0	6½	15,300	94	5	5.88 @ 20.6 C.	2.13 @ 22.8 C.
24 hours	6,180,000	32.0	4	8,400	82	16	5.40 @ 20.8 C.	2.10 @ 22.8 C.
48 hours	5,870,000	38.0	9	18,200	92	5	4.38 @ 21.0 C.	1.85 @ 22.9 C.
72 hours	6,200,000	24.0	4	16,100	83	15	4.76 @ 21.2 C.	1.87 @ 23.0 C.
7 days	6,860,000	21.0	10	22,200	95	3	4.60 @ 23.0 C.	2.04 @ 21.2 C.
9 days	6,670,000	19.0	10	23,900	95	3	4.90 @ 23.2 C.	2.09 @ 21.3 C.
14 days	6,050,000	1.0	12	12,800	98	2	4.94 @ 21.4 C.	1.90 @ 22.3 C.

(0.75 cc. of lime water to 5 cc. of a 1.2 per cent solution of pectin in physiologic solution of sodium chloride) or phosphate buffer solution.

Hematic Reactions.—Single or repeated injections of freshly prepared 1 per cent or 2 per cent pectin solutions were made into the jugular veins of dogs for the study of the hematic effects. In the single injection series 3 dogs weighing about 7 Kg. each were given intravenously 10 cc., 20 cc. and 35 cc., respectively, of a 1 per cent neutralized pectin solution. The procedure followed and the results obtained are presented in table 1, containing the data on the dog which received the medium dose and showed moderately marked hematic effects.

2. Dehn, W. M., cited by Elwell.[7]

3. Myers, P. B., and Baker, G. L.: Fruit Jellies: VIII. The Role of Pectin; 4. The Physico-Chemical Properties of Pectin, Bulletin 187, Technical no. 15, University of Delaware Agricultural Experiment Station, Newark, Delaware, 1934.

4. Hinton, C. L.: Fruit Pectins: Their Chemical Behavior and Jellying Properties, Special Report 48, Department of Scientific and Industrial Research: Food Investigation, New York, Chemical Publishing Company, Inc., 1940.

5. Joseph, G. H.: Bull. Nat. Formulary Committee **9**:2 and 18, 1940.

Immediately following the injection of the pectin solution there occurred a brief transitory increase in the number of erythrocytes per cubic millimeter followed by a drop to somewhat below the original level. This movement was reversed within the first twenty-four hours for the 2 dogs which received the small and the medium-sized dose, but was progressive for the dog which was given 35 cc. of a 1 per cent pectin solution, so that at the end of the observation period of fourteen days the initial number of 4,300,000 was reduced to 1,700,000. The hemoglobin values and the amounts of packed erythrocytes followed in general the fluctuations of the erythrocyte counts. The sedimentation rate of the erythrocytes was markedly increased after the injection. The rate returned to normal or almost normal at about eight hours after the treatment to reach again high values within the next twelve hours and to remain high for another six to eight days. There was gradual slowing of the erythrocytic sedimentation afterward, with return to normal conditions at the termination of the fourteen day period. While the prothrombin time remained stationary, the coagulation time was in general moderately shortened immediately after the injection. This reaction lasted for several days, after which a return to normalcy was observed.

There developed a progressive decrease in the viscosity of the whole blood, although no such alteration was demonstrable concerning the viscosity of the plasma. This movement was still active at the end of the observation period. The leukocytes dropped sharply and markedly after the injection of the pectin solution in the 2 dogs which received 20 and 35 cc. of this material, while no change occurred in the dog which received only 10 cc. The leukopenic reaction lasted for about six hours. During this phase there was marked diminution in the percentage of the polymorphonuclear leukocytes with a relative increase of the lymphocytes.

Of the 3 dogs used for the study of the effects of repeated injections of pectin solution, 2 belonged to the former series. They received daily five times per week 20 cc. of a 1 per cent solution. After six weeks the daily dose was increased to 30 cc., and after eight weeks the concentration was increased to 2 per cent. During the thirteenth week the daily dose was raised to 40 cc., and during the following and last week of the experiment, to 55 cc. A total of 25 Gm. of pectin was injected. Five days after the last injection the animals were killed. The hematic effects observed are presented in table 2, which contains the data on one of the dogs. These data are characteristic for the entire series.

During the first two weeks of the experiment there was in 1 animal a moderate increase in the number of erythrocytes, while the other 2 dogs showed a moderate drop followed by a brief rise. A mild to moderate reduction in the number of red cells occurred toward the end of the experimental period. The hemoglobin content and the volume of packed erythrocytes followed the trend exhibited by the number of red cells. The sedimentation rate was consistently increased. The prothrombin time fluctuated within normal limits. The coagulation time exhibited at times relatively low values. The number of leukocytes as well as their ratio did not undergo any appreciable changes. Mononuclear leukocytes with basket weave vacuolated cytoplasm were found in small number. The viscosity of the whole blood followed in general the fluctuations in the number of erythrocytes, while the viscosity of the plasma remained within normal limits.

It appeared to be desirable to investigate also the hematic reactions elicited by pectin solutions that had been subjected to autoclaving, as pectin solutions thus treated had been proposed as blood substitutes (Hartman and co-workers [6]).

6. Hartman, F. W.; Schelling, V.; Harkins, H. N., and Brush, B.: Ann. Surg. **114**:212, 1941.

TABLE 2.—*Hematic Reactions in Dog 797, Weighing 6.3 Kg., After Intravenous Administration of, First, 710 Cc. of a 1 per Cent Pectin Solution and, Second, 875 Cc. of a 2 per Cent Pectin Solution*

	Amount of Pectin, Cc.	Erythrocytes per Cu. Mm.	Sedimentation Rate, Mm.	Coagulation Time, Min.	Leukocytes, per Cu. Mm.	Polymorphonuclears, per Cent	Lymphocytes, per Cent	Viscosity	
								Whole Blood	Plasma
Start	.....	6,050,000	1.0	12	12,800	98	2	4.94 @ 21.4 C.	1.90 @ 22.3 C.
9 days	120 × 1%	7,210,000	21.0	7	17,400	84	15	5.12 @ 20.4 C.	1.79 @ 18.2 C.
14 days	100 × 1%	7,650,000	3.0	12	14,400	82	16	7.60 @ 20.6 C.	2.06 @ 22.6 C.
21 days	100 × 1%	6,730,000	13.0	6½	11,500	80	19	6.18 @ 19.5 C.	1.92 @ 23.2 C.
28 days	100 × 1%	6,680,000	11.0	4	9,500	78	11	6.40 @ 21.0 C.	2.06 @ 19.5 C.
35 days	100 × 1%	5,770,000	44.0	13	14,200	84	12	4.66 @ 20.8 C.	1.88 @ 18.2 C.
56 days	190 × 1%	6,200,000	31.0	17	10,200	86	8	6.32 @ 18.8 C.	2.36 @ 18.7 C.
	190 × 2%								
77 days	685 × 2%	5,900,000	40.0	11	20,000	84	10	5.80 @ 18.2 C.	2.66 @ 20.7 C.

Previous investigators (Myers and Baker[3]; Elwell[7]; Bryant[8]; Joseph[5]) had shown that exposure of pectin solutions to heat causes relatively rapid reduction in viscosity and in the size of the pectin molecule (depolymerization), resulting in the formation of a brown flocculent precipitate. Bryant[8] reported that the autoclaving of a pectin solution, consisting of molecules having a molecular weight of approximately 225,000, causes a reduction of the molecular weight after fifteen minutes to 75,000, after thirty minutes to 30,000 and after two hours to 3,000.

Similar observations were made when the pectin solutions used in the experiments reported here were adjusted by the addition of lime water or of phosphate buffer solution to the neutral point and then autoclaved for ten or twenty minutes at 8 or 15 pounds' (3.5 or 7 Kg.) pressure. The viscosity dropped from the original value of 4.96 at 21 C. to 1.2, while a flocculent precipitate was formed in the dark amber liquid having a caramel-like odor. The formation of a precipitate caused a lowering of the osmotic pressure (23 cm. of water). It was demonstrated that there was a similar marked drop in osmotic pressure when flocculation developed in a freshly prepared, nonautoclaved pectin solution left for twenty-four hours in a collodion bag during osmotic pressure experiments. Heated pectin solutions therefore have physicochemical properties differing markedly from those of fresh, unheated ones.

The heated pectin solutions used in the experiment were obtained as follows: The 1.2 per cent pectin solution prepared as described in an earlier paragraph was autoclaved at 15 pounds for twenty minutes. After cooling it was filtered through a Büchner filter and Whatman no. 1 filter paper for the removal of the precipitate formed. It was then resterilized under the same conditions as before. A buffer salt solution (9 Gm. of sodium chloride, 1 Gm. of potassium dihydrogen phosphate, 6 Gm. of disodium acid phosphate and 250 cc. of water) was sterilized separately. For injection purposes three parts of the pectin solution were mixed with one part of the buffer salt solution, giving a mixture of an almost neutral reaction (p_H 6.5). For the preparation of a neutralized 2 per cent pectin solution the amount of sodium phosphate was doubled (12 Gm.). The sterilized, nonneutralized pectin solution is unstable and must be prepared freshly, as on incubation in the ice box it forms a slight fine grayish white precipitate. The amount of this precipitate is moderately increased when neutralized, sterilized pectin solutions are kept in the ice box.

In the hematologic studies with the heated pectin solution 3 dogs were used, which were treated similarly to those in the previous experiment. Table 3 lists the hematic reactions observed in 1 dog after a single intravenous injection of 27 cc. of a sterilized 1 per cent pectin solution. These reactions were characteristic of the entire series.

The results are similar to those obtained with the unsterilized pectin solution. The effect on the number of erythrocytes during the first seventy-two hours is striking. There is also definite and progressive lowering of the viscosity of the whole blood.

Table 4 presents the data obtained after repeated intravenous injections of 1 per cent and 2 per cent sterilized pectin solution.

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7. Elwell, W. E.: Pectin: Its Manufacture, Properties and Uses, Seattle, University of Washington, State W. P. A. Project, 1939.

8. Bryant, E. F.; Palmer, G. H., and Joseph, G. H.: Proc. Soc. Exper. Biol. & Med. **49**:279, 1942.

It is evident that this treatment exerts mainly only a hastening effect on the sedimentation rate, leaving the number of erythrocytes, the amount of hemoglobin, the volume of packed red cells, the number and the ratio of leukocytes and the viscosity of the whole blood and of the plasma without appreciable changes.

Organic Reactions.—The changes effected in the various tissues and organs by the repeated intravenous injection of autoclaved and nonautoclaved pectin solution were studied in 7 dogs and 12 rabbits. Four dogs and 6 rabbits were given nonautoclaved pectin solution, and 3 dogs and 6 rabbits were treated with autoclaved pectin solution.

Of the 4 dogs given injections of nonautoclaved pectin solution, 3 were used in the hematic study. The weights of these animals at the start and at the end of the experiment, the total amount of pectin solution administered and the duration of the experiment are given in table 5.

TABLE 3.—*Hematic Reactions in Dog 805, Weighing 6.4 Kg., After a Single Intravenous Administration of 27 Cc. of a 1 per Cent Buffered Sterilized Pectin Solution*

	Erythrocytes per Cu. Mm.	Sedimentation Rate, Mm.	Coagulation Time, Min.	Leukocytes per Cu. Mm.	Polymorphonuclears, per Cent	Lymphocytes, per Cent	Viscosity	
							Whole Blood	Plasma
Start	6,010,000	14	11	10,700	88	9	6.06 @ 16.0 C.	2.00 @ 21.5 C.
5 minutes	6,040,000	46	18	4,600	80	17	5.92 @ 16.5 C.	2.00 @ 21.6 C.
15 minutes	6,000,000	37	9½	8,800	81	14	6.08 @ 17.0 C.	1.98 @ 21.8 C.
45 minutes	5,890,000	41	8	10,900	78	11		
2 hours	5,460,000	28	6	11,100	89	8		
4 hours	5,560,000	36	6	13,700	81	8	5.36 @ 17.4 C.	1.90 @ 21.7 C.
6 hours	5,080,000	4	12½	12,800	80	10		
8 hours	5,020,000	2	13	12,900	80	13		
24 hours	5,050,000	2	4	9,900	87	10	4.92 @ 18.0 C.	1.80 @ 21.4 C.
48 hours	5,180,000	33	7	8,900	71	18	4.32 @ 18.3 C.	1.81 @ 21.4 C.
72 hours	5,080,000	18	5	10,900	84	8		
7 days	5,760,000	20	11	20,900	79	18		
10 days	5,520,000	39	9	21,600	87	10	4.84 @ 21.4 C.	1.86 @ 19.7 C.
14 days	5,410,000	15	.....	17,700	72	23	6.06 @ 19.0 C.	2.13 @ 22.2 C.

The dogs were killed at the end of the experimental period.

The 6 rabbits of this experiment received in the beginning 10 cc. of a 1 per cent solution five times a week. This dose was gradually increased to 30 cc. daily, and the concentration was doubled. Four of the rabbits died after the thirteenth, fourteenth, fifteenth and sixteenth injection, respectively, while the surviving 2 rabbits were put to death after forty-nine injections. The total amount given varied for the individual animal from a minimum of 330 cc. to a maximum of 1,380 cc. of pectin solution, delivered within three to twelve weeks.

The injections were tolerated by the dogs and rabbits without any immediate untoward effects. It was noted in the rabbits, however, that the aural veins which were used for injection became gradually obliterated and that because of this complication 4 of the rabbits had drooping, swollen, purplish red ears.

At postmortem examination the spleens of the 4 rabbits which died early during the experiment were about four times normal size, pale grayish red and relatively soft. The livers were enlarged and had dark brown-red surfaces showing light grayish spotting or were grayish brown. Multiple dark red indurations were present in the lungs.

TABLE 4.—*Hematic Reactions in Dog 805, Weighing 6.4 Kg., After Repeated Intravenous Administration of 550 Cc. of 1 per Cent and 875 Cc. of 2 per Cent Buffered Sterilized Pectin Solution*

	Amount of Pectin, Cc.	Erythrocytes per Cu. Mm.	Sedimentation Rate, Mm.	Coagulation Time, Min.	Leukocytes, per Cu. Mm.	Polymorphonuclears, per Cent	Lymphocytes, per Cent	Viscosity	
								Whole Blood	Plasma
Start	.....	5,410,000	15	.....	17,700	72	23	6.06 @ 19.0 C.	2.13 @ 22.2 C.
3 days	60 × 1%	6,040,000	4	9	17,300	76	17	6.00 @ 20.0 C.	2.12 @ 22.6 C.
10 days	100 × 1%	5,630,000	29	4½ scc.	20,100	91	4	5.56 @ 18.3 C.	1.95 @ 22.8 C.
17 days	100 × 1%	5,260,000	3	13	16,900	76	16	5.38 @ 20.2 C.	1.94 @ 18.8 C.
24 days	100 × 1%	5,680,000	19	9½	14,100	77	16	4.85 @ 20.2 C.	1.76 @ 17.5 C.
45 days	190 × 1%	5,660,000	46	7½	11,700	71	23	5.65 @ 18.5 C.	2.00 @ 18.4 C.
	190 × 2%								
56 days	685 × 2%	6,300,000	7	11½	14,400	67	26	6.08 @ 17.5 C.	1.64 @ 20.1 C.

In the 2 rabbits and 4 dogs which were killed at the end of the experimental period the spleens were moderately enlarged. The livers were normal in appearance and color. The lymph nodes were somewhat enlarged. All other organs were normal in size, consistency and color.

The following observations were made on microscopic examination:

A few to numerous pulmonary precapillary and capillary vessels were filled by a stringy refringent homogeneous unstained or faintly bluish-stained material, which was surrounded by proliferated endothelial cells and multinucleated giant cells or by a multinucleated syncytial mass. Large polypous accumulations of giant cells or polypous granulomatous lesions consisting of giant cells and foam cells in a mucinous matrix projected into the lumens of several small or medium-sized vessels (fig. 1 *A*). Some of the precapillary vessels had markedly thickened hyaline walls, while others were infiltrated and surrounded by leukocytes and lymphocytes. Small giant cell nodules enclosing globules of homogeneous matter were found in the perivascular tissue. The endothelial lining of the pulmonary artery of 1 dog showed foam-cellular transformation and cushion-like thickenings.

In some animals the myocardium had a loose granular cytoplasmic texture. The myocardial arterioles often had thickened hyaline walls, while larger arteries

TABLE 5.—*Data on Dogs Given Injections of Nonautoclaved Pectin Solution*

Dog	Weight, Kg.		Amount of Pectin Solution, Cc.	Duration, Weeks
	At Start	At End		
779	7.1	7.5	1,635	12
797	6.3	6.0	1,605	12
821	9.0	8.8	1,505	10
823	7.8	5.9	400	4

showed hyaline swelling of the media, in which swollen, irregularly arranged and distorted nuclei were embedded (fig. 1 *B*). In several animals there were foam-cellular transformation and thickening of the epicardial and myocardial coronary vessels, occasionally associated with foam cellular infiltration of the media. In others the endothelial lining exhibited edematous swelling, resulting in a scalloped appearance of the intima.

In 2 rabbits the endothelial lining of the ascending portion of the aorta consisted of a single layer of cuboidal foam cells. This was associated in places with diffuse "mucinous" imbibition of the inner media. The muscle cells in such areas were swollen and had loose, almost unstained cytoplasm. In all the dogs the aorta exhibited smaller or larger foci of endothelial and often foam-cellular proliferation. The smallest lesions consisted of groups of three or four crowded endothelial cells, possessing in places foamy, swollen cytoplasm. Somewhat larger ones formed small shallow elevations consisting of densely packed cells with small oval nuclei (fig. 2 *A* and *B*). There were large foam-cellular cushion-like intimal thickenings in other places (fig. 2 *C*). The foam-cellular accumulations were occasionally eroded on the surface and infiltrated with dark oval-shaped cells. In larger lesions both types of cells freely invaded the edematous subintimal media and were found in and around the vasa vasorum. A narrow hyaline necrotic zone was occasionally found in the subintima underneath some of the foam-cellular accumulations. Older lesions showed more or less complete hyalinization of the intimal thickenings, which in a few instances also contained larger areas of calcifi-

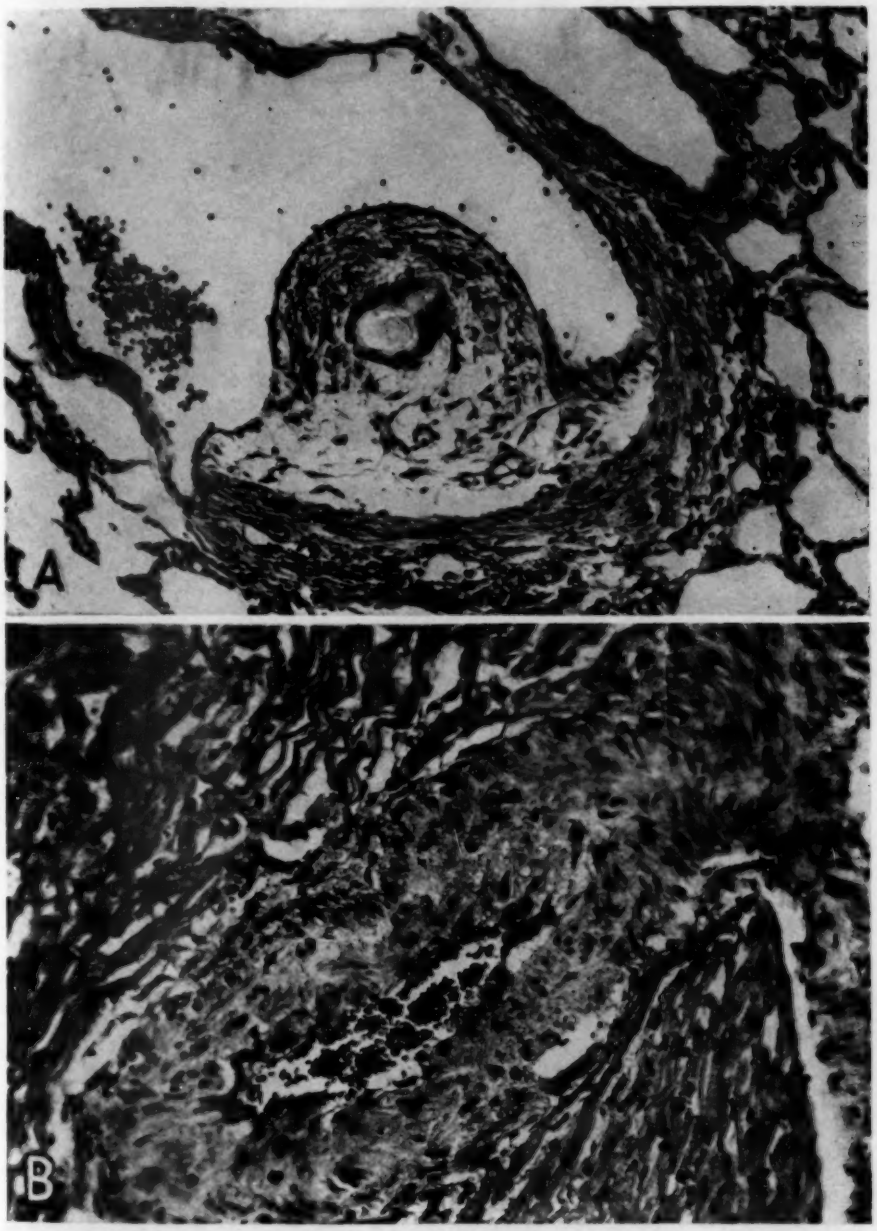

Fig. 1.—*A*, pulmonary vessel with polypous foam-cellular and giant-cellular granuloma of the intima; × 150. *B*, myocardial arteriole with swollen and partly hyalinized media containing irregularly distributed round nuclei; × 200.

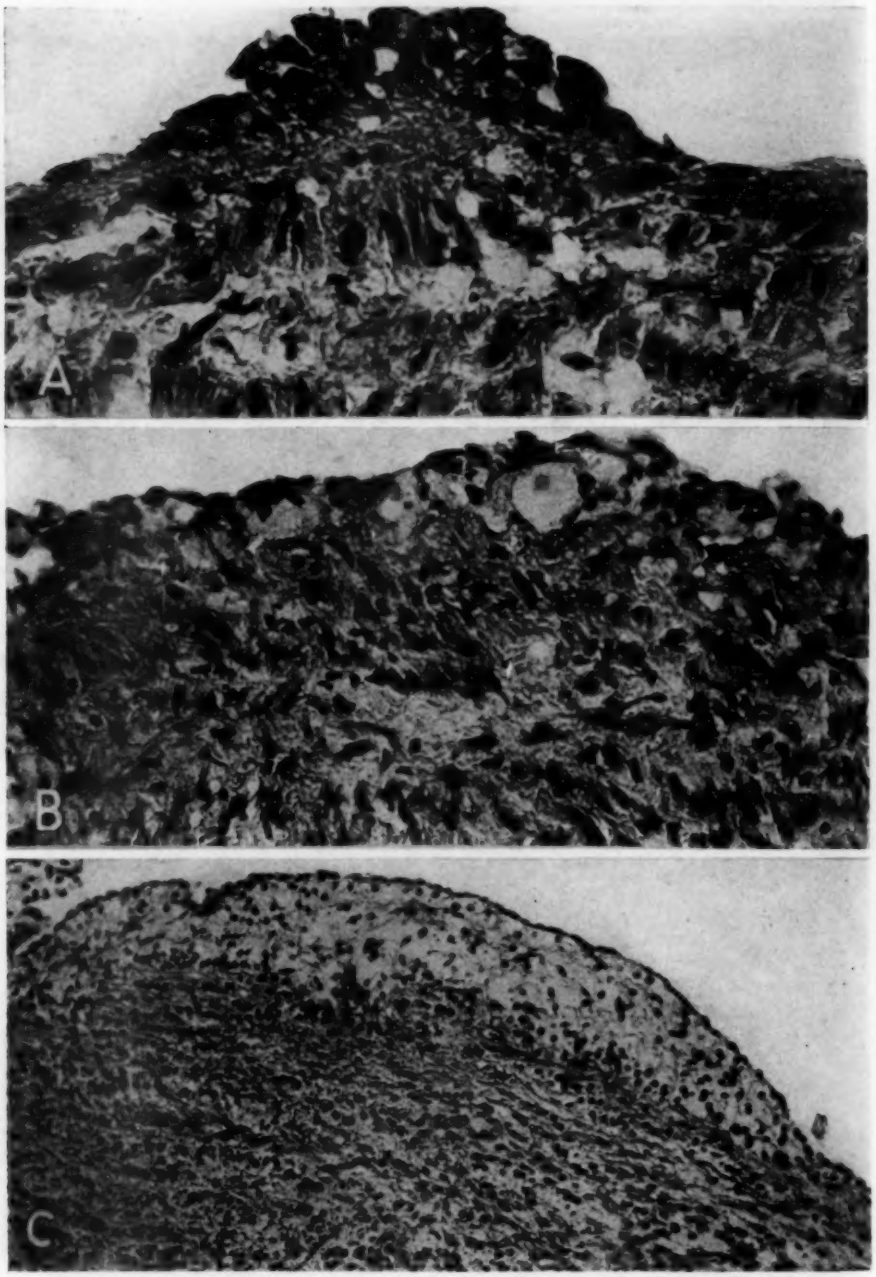

Fig. 2.—*A*, aorta with a small cone-shaped proliferation of the endothelial cells; × 700. *B*, aorta with multiple foci of endothelial proliferation and transformation of endothelial cells into foam cells; × 200. *C*, cushion-like foam-cellular thickening of the intima with beginning invasion of the media; × 150.

cation and of fibrous transformation. Larger areas of hyaline degeneration of the deeper parts of the media were found in several instances (fig. 3 *A*).

Similar lesions were present, though less frequently and extensively, in the arteries branching from the aorta.

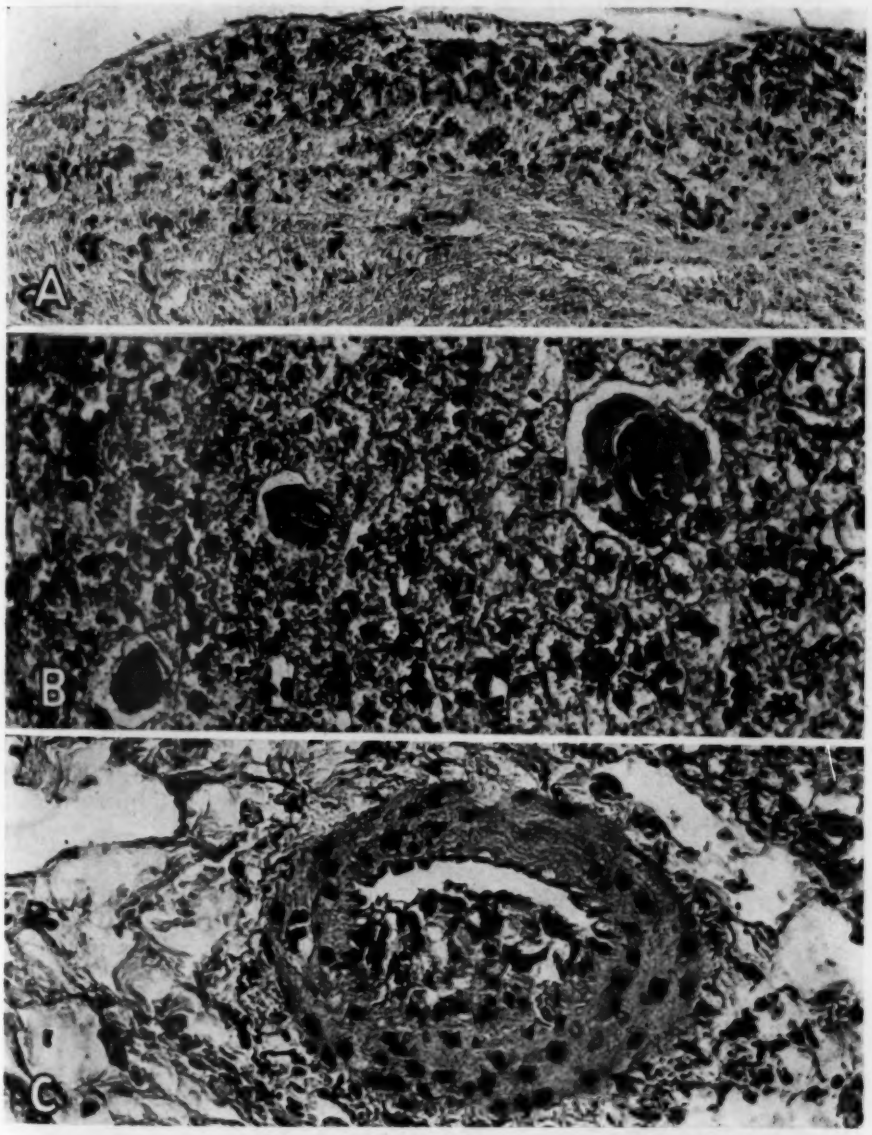

Fig. 3.—*A*, hyaline thickening of the aortic intima with diffuse calcium incrustations; × 150. *B*, liver with foam type liver cells and focal accumulations of Kupffer cells, some of which have the character of multinucleated giant cells; × 350. *C*, small cushion-like thickening of the intima of a hepatic artery; × 180.

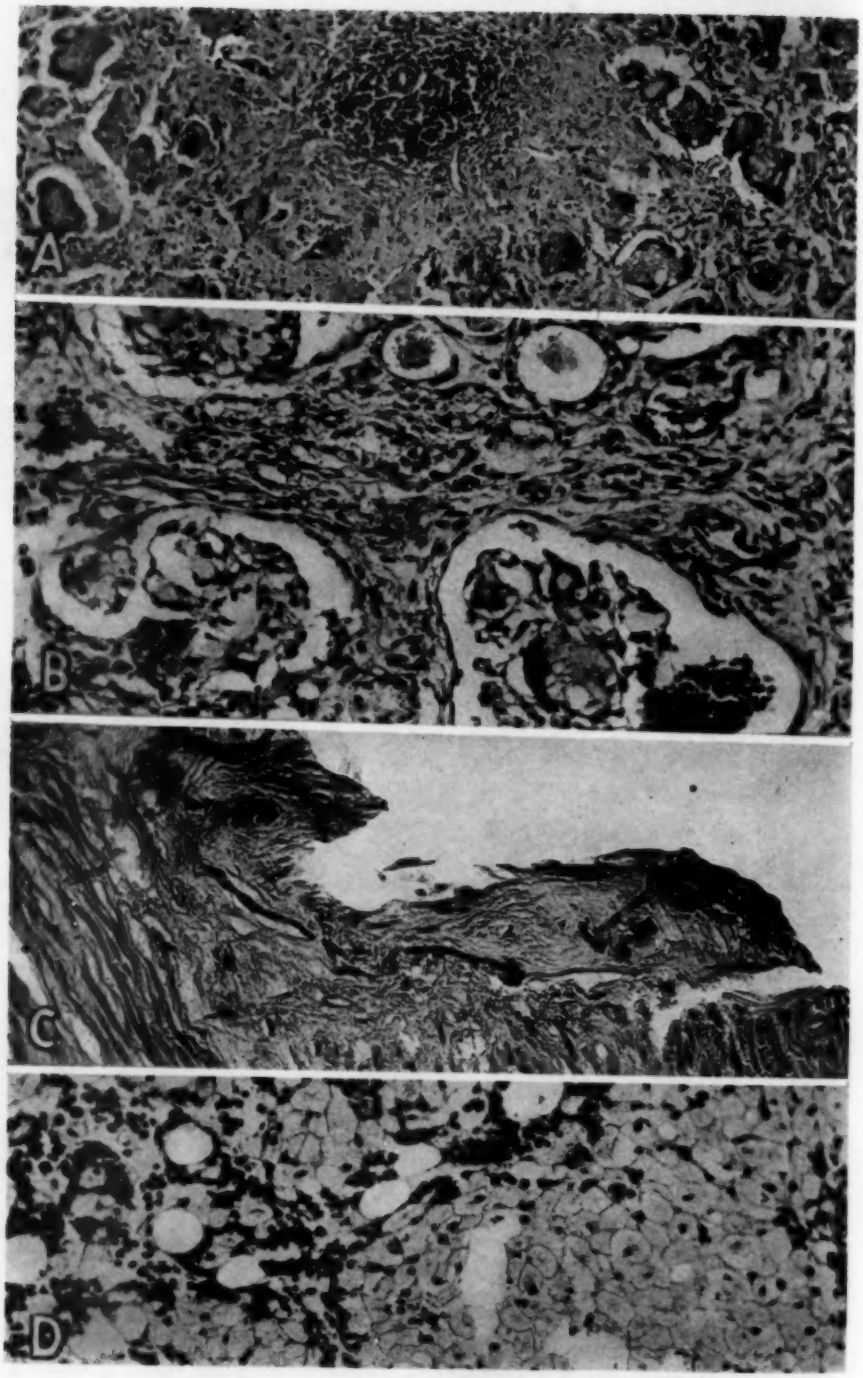

Figure 4

(See legend on opposite page)

The thyroid and parathyroid glands were normal.

The hepatic cells in the livers of the different animals showed varying degrees of foam-cellular transformation (fig. 3 *B*). This change was particularly marked in the rabbits, while in the dogs the cytoplasm of these cells had a flaky appearance. Similar differences occurred in the participation of the Kupffer cells in the storage process. These cells in the rabbits were in general swollen, proliferated and not infrequently changed into multinucleated giant cells, which occurred occasionally also in the periportal connective tissue. Giant type and foam type Kupffer cells, on the other hand, were infrequent in the livers of the dogs. Branches of the hepatic artery occasionally contained small cushion-like proliferations of endothelial cells, which had partly a foamy cytoplasm (fig. 3 *C*).

In the 4 rabbits which died after thirteen to sixteen injections the pulp of the spleen was replaced by large conglomerations of huge multinucleated giant cells and foam cells embedded in a homogeneous and stringy material (fig. 4 *A*). Atrophic lymph follicles were surrounded by zones of hyaline necrosis, in the periphery of which were found ill defined syncytial masses with their nuclei arranged in a semicircle. Similar but less extensive lesions were present in 1 dog. In the 2 rabbits which were killed at the end of the experiment as well as in 3 dogs the spleen was hyperplastic and contained a varying amount of brown pigment and sometimes a scattering of foam cells.

Apart from general moderate hyperplasia, there was no pathologic change in the lymph nodes.

The stomach and intestine were normal in all animals except 1 rabbit, in which the small intestine contained several mucosal hemorrhages associated with greenish gray-tinted foam cell infiltrations in the submucosa.

The pancreas showed no abnormalities.

In several animals the reticulum cells of the adrenal medulla were enlarged and possessed foamy cytoplasm. However, this change remained always within moderate limits.

In the kidneys of the rabbits and of 2 of the dogs the glomeruli possessed in varying degrees a grayish purple homogeneous matrix and showed swollen, foam type endothelial cells and multinucleated giant cells (fig. 4 *B*). Thickening of Bowman's capsule, which was occasionally adherent to the grapelike transformed glomerulus, was seen in places. Erythrocytes and hyaline material were found in the capsular spaces. The interstitial tissue of the cortex was diffusely thickened in several animals and was infiltrated with round cells and foam cells. The tubules were in general normal. The arterioles of the canine kidneys often showed endothelial proliferation and hyalinization in the form of shallow or polypous bulges projecting into the lumens. Other arterioles exhibited medial hypertrophy with focal hyalinization. The main renal artery of 1 dog showed marked hyaline intimal thickening with calcium incrustation (fig. 4 *C*).

EXPLANATION OF FIGURE 4

A, spleen with marked atrophy of the lymph follicle, perifollicular hyalinization and numerous multinucleated giant cells and foam cells in the pulp; × 150. *B*, glomeruli with an edematous interstitial tissue, foam type endothelial cells and multinucleated giant cells; × 275. *C*, renal artery with hyaline and calcified thickening of the intima; × 275. *D*, foam-cellular transformation and proliferation of the reticulum cells of the bone marrow; × 250.

The bone marrow of the sternum in the rabbits was a loose structure which was permeated by strands and clusters of large foam cells of reticulum cell origin (fig. 4 *D*). These changes were absent in 3 dogs and were only mild in the fourth. The myeloid elements were mainly of the immature type.

No lesions were found in the cerebral parenchyma, the vascular system of the brain or the choroid plexus.

The anterior portion of the hypophysis contained numerous eosinophilic cells. Some of the pale grayish blue–stained cells were swollen and ballon-like and had foamy cytoplasm.

Many of the sections were stained with ruthenium red for the histochemical demonstration of pectin (Tobler [9]). Large amounts of pectin were visualized by this method as cherry red–stained material in the distended pulmonary capillaries. Reddish granules were found in the foam-cellular intima of the aorta as well as in the foam type liver cells and reticulum cells of the adrenal. The pectin retained in the Kupffer cells was globular or represented a larger solid mass. The giant cells of the spleen were filled with similar masses from which a stringy red–stained network radiated into the sinusoidal spaces. The glomerular capillaries and endothelial cells were filled with reddish globules. Solid casts of

TABLE 6.—*Data on Dogs Given Repeated Injections of an Autoclaved Pectin Solution*

Dog	Weight, Kg.		Amount of Pectin Solution, Cc.	Duration, Weeks
	At Start	At End		
796	7.5	10.75	1,475	11
805	6.4	4.5	1,450	9
845	6.0	4.5	430	4

deep red–stained matter were present in the tubular lumens. The foam type reticulum cells of the bone marrow gave a striking reaction for red-stained globular matter.

Sections of the various organs, especially the liver, the spleen and the aorta, stained for fat substances with sudan III, were practically free from such substances. Only a few orange red granules were visible in a few of the splenic giant cells.

A similar pathologic study was made of 3 dogs and 6 rabbits which were given five injections a week of an autoclaved pectin solution, prepared as described in an earlier paragraph. The 6 rabbits, weighing about 3 Kg. each, received daily 35 cc. of a 2 per cent autoclaved pectin solution for a period of fifteen weeks (74 injections with a total of 2,520 cc. of injected solution). The injections were tolerated well. The rabbits were killed at the end of the experimental period. The autopsy showed that 3 had markedly enlarged spleens. All other organs were grossly normal.

Two of the dogs used in this investigation were those used for the hematic studies. Table 6 gives the experimental data of this series.

As dog 845 became rapidly anemic under the treatment and lost considerable weight (1.5 Kg. in four weeks), it was killed after four weeks of treatment. The autopsy showed a highly edematous pancreas and atelectatic areas in the lungs. The postmortem observations on the other two dogs of this series were normal.

9. Tobler, F.: Ztschr. f. wissensch. Mikr. **23**:182, 1906.

The histologic examination of the tissues of the animals revealed the following lesions:

One of the 6 rabbits exhibited the fully developed picture of atheromatosis of the aorta and intimal thickenings with medial degeneration of the renal and pulmonary arteries and capillaries as well as the thesaurosis of the internal organs (giant cell granulomatous lesions in the splenic pulp, foam-cellular transformation of liver cells and reticulum cells of bone marrow). In the other 5 rabbits the reactions elicited were limited to accumulations of foam cells in the bone marrow and to minor foci of intimal thickening and medial hyaline degeneration of the pulmonary and renal arteries and of the aorta.

In the 3 dogs only mild edematous changes in the aortic wall were observed. The intima had in places a garland-like outline caused by the presence of small accumulations of round or cuboidal cells underneath balloon-like swollen endothelial cells. Similar edematous vascular reactions occurred occasionally in smaller vessels of the elastic type. There was no evidence of a thesaurosis in any of the organs studied.

COMMENT

The data presented indicate that the injection of nonautoclaved, freshly prepared solutions of the hydrophilic colloid pectin elicits reactions in the blood and in the tissues of the internal organs which are similar to or identical with those caused by other macromolecular colloidal agents (colloidoclastic leukopenia, accelerated erythrocytic sedimention, reduction of erythrocytes, atheromatosis, thesaurosis). The shortening of the coagulation time noted in these animals, which is in contrast to observations with other macromolecular agents (polyvinyl alcohol, methyl cellulose), represents apparently a specific action of the acid groups contained in the pectin molecule and confirms the statements made by previous investigators concerning the hemostatic effect of pectin (Violle and Saint-Rat [10]; Riesser and Nagel [11]; Sack [12]; Gohrbandt [13]; Derouaux [14]; Riesser [15]; Baumann [16]; Feissly [17]; Ziegelmayer [18]).

The lack of prolonged clotting time and of increased plasmatic viscosity even after prolonged treatment with highly viscous pectin solutions is an indication of the relative instability of this substance in the blood.

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10. Violle, H., and de Saint-Rat, L.: Compt. rend. Acad. d. sc. **180**:603, 1925; Compt. rend. Soc. de biol. **92**:1097, 1924.
11. Riesser, O., and Nagel, A.: Arch. f. exper. Path. u. Pharmakol. **179**:748, 1935.
12. Sack, G.: Klin. Wchnschr. **14**:1536, 1935.
13. Gohrbandt, E.: Deutsche med. Wchnschr. **62**:1625, 1936.
14. Derouaux, G.: Arch. internat. de pharmacodyn. et de thérap. **62**:100, 1939.
15. Riesser, O.: Klin. Wchnschr. **14**:958, 1935.
16. Baumann, E.: Beitr. z. klin. Chir. **166**:298, 1937.
17. Feissly, R.: Compt. rend. Soc. de biol. **92**:317, 1925.
18. Ziegelmayer, W.: Kolloid-Ztschr. **71**:214, 1935.

This fact apparently also accounts at least in part for the absence of any appreciable and persistent myeloid reactions in the blood of the dogs treated by repeated injection of pectin solutions, such as those seen after the repeated intravenous introduction of polyvinyl alcohol and methyl cellulose. The retention of pectin in the reticulum cells of the bone marrow may be active in the same direction, by interfering with the proliferative activity of the bone marrow.

The storage phenomena in the liver, spleen, kidney and bone marrow are, on the other hand, evidence that that part of the pectin which is taken up within cells is of higher stability or is protected by the conditions prevailing in the cytoplasm against the rapidly disintegrative influences operative in the blood. The morphologic manifestations characterizing these thesaurotic reactions bear a marked similarity to certain lipoidoses. Thus, the foam-cellular pectinic transformations of the reticulum cells of the bone marrow resemble similar lipoidal changes associated with Schüller-Christian disease. In this respect it is remarkable that the rabbits showed the various storage phenomena much more highly developed than the dogs. Differences in relative dose of pectin introduced may account in part for these discrepancies.

Mention must be made, however, of the fact, that the thesaurosis was most marked in the 4 rabbits which died early in the experiment, while it was less pronounced in the 2 rabbits which survived longest and therefore received the largest total dose. Similar observations were made in connection with experiments on methyl cellulose thesaurosis, in which the dogs which died spontaneously after a relatively short time in the experiment revealed the most extensive splenic necrosis with numerous giant cell granulomatous lesions identical with those seen in the 4 pectin-treated rabbits. Species-specific and individual metabolic differences therefore may contribute to the variations in the degree of thesaurosis elicited by various colloidal storage substances.

The observations concerning the different developmental stages of pectin atheromatosis leave no doubt that the first sign of the formation of atheroma is a proliferation and swelling of a few endothelial cells. With further development of the multiple small seedlike lesions there occurs progressive proliferation and foam-cellular transformation of the endothelial cells, producing smaller and larger intimal thickenings. Foam cells from these foci may later invade the edematous inner media, while at the same time endothelial cells of the vasa vasorum may take up pectin and be thereby transformed into foam cells. There may occur subsequently a breakdown of the atheromatous tissues, with the development of erosions and the appearance of small round or oval cells with dark-stained round nuclei within the atheromatous lesions and the surrounding media. In other instances, the necrosis of the foam cells

is followed by calcification. Hyaline transformation of the intimal thickenings with or without larger calcium incrustations may follow ultimately. During these intimal developments, the superficial media may undergo degenerative changes which assume the shape of a hyaline band underneath the atheromatous lesions. Larger areas of hyaline degeneration and calcification are found later on in the middle zone of the media.

In addition to these focal alterations, the arterial intima sometimes exhibits more diffuse foam-cellular transformation. Although similar intimal lesions are observed occasionally in the pulmonary vessels, changes typical of this organ are giant cell granulomatous lesions of the intima, such as those previously described in animals given injections of polyvinyl alcohol, methyl cellulose or emulsions of cholesterol. The renal, myocardial and hepatic arterioles, on the other hand, show proliferative and degenerative intimal and medial lesions which are similar to those seen in the large elastic arteries.

While blood smears show the presence of vacuolated mononuclear cells in the circulating blood, there is no evidence that these participate significantly in the production of the atheroma.

There seems to be little doubt that the atheromatous formations produced by the retention of pectin in the endothelial cells of the arterial intima are of less permanence than those elicited by polyvinyl alcohol, methyl cellulose or cholesterol. The data available indicate that the foam-cellular intimal proliferations lose relatively rapidly their pectin content and undergo either hyaline necrosis or fibroblastic organization. Thus, primary atheromatous lesions are transformed into secondary sclerotic lesions. While observations made in animals given injections of polyvinyl alcohol or methyl cellulose (unpublished experiments) suggested the occurrence of such transitions, which are known in connection with cholesterol atheromatosis, the data presented in regard to pectin atheromatosis show that the speed of occurrence of such secondary changes depends on the metabolic stability of the causal atheromatogenic substance.

The capillaries of the renal glomeruli exhibit lesions such as are found in connection with the intravenous introduction of polyvinyl alcohol, methyl cellulose, acacia, ovalbumin-azoprotein, ovalbumin and gelatin, and resemble those seen in certain types of human glomerulitis. They apparently represent reactions to disturbances in glomerular filtration and are elicited by the retention of pathologic macromolecules circulating in the plasma in the glomerular capillaries.

The validity of these conclusions is supported by the observations made in the animals given injections of the autoclaved pectin solution as well as those reported previously by others (Myers and Baker [8];

Joseph [5]; Hinton [4]; Elwell [7]; Bryant, Palmer and Joseph [8]; Bonner [19]). As it was shown that heating pectin solution causes hydrolysis of the pectin molecule and thus its depolymerization, it is significant that the animals treated with the autoclaved pectin solution showed, with the exception of 1 rabbit, an almost complete lack of storage phenomena in the internal organs and the presence of only minor vascular degenerative changes, among which typical foam-cellular lesions were strikingly absent. These findings indicate that the marked reduction in the molecular weight and size of the pectin molecule produced by exposure to prolonged heat resulted in increased and relatively rapid excretion of the injected macromolecular material or in ready progressive metabolic degradation to still smaller molecular units penetrating easily the filtration membranes. It is thus demonstrated that the production of atheromatosis and thesaurosis by the injection of pathogenic macromolecular substances depends on the size of the molecules and on the metabolic stability of these substances.

Inasmuch as autoclaved pectin solutions have been proposed as nonhematogenous blood substitutes in the treatment of shock (Hartman and co-workers [6]; Bryant and co-workers [8]), these observations deserve special attention. It is obvious that both groups of investigators, particularly Hartman and co-workers, employed a solution that contained essentially degradation products of pectin which possessed a molecular weight definitely below that of the smallest plasma proteins. It stands to reason that molecules of this type will penetrate the injured and highly permeable filtration membranes of shocked persons almost as readily as the molecules of crystalloid solutions, and are presumably just as effective as these. Attention must be called to the fact that the concentration of pectin in autoclaved solutions is markedly lower than that in the original solution before sterilization, as a considerable amount of precipitate must be removed from an autoclaved solution before it becomes suitable for intravenous medication. If one considers that pectin solutions are relatively acid (p_H 3.2) and that the difficulties connected with their preparation are accentuated when such solutions are neutralized, it becomes evident that it is practically impossible to prepare a sterilized and standardized product of neutral reaction which consists of pectin molecules of sufficient size and which does not deteriorate rapidly into a solution of low molecular degradation products under the formation of a precipitate.

It may be pointed out in this connection that the effectiveness of colloid therapy for shock, regardless of the type of colloid used, does not depend primarily on the osmotic pressure exerted by a particular colloidal solution, as any effects depending on such action are more

19. Bonner, J.: Bot. Rev. **2**:475, 1936.

readily obtainable by the introduction of crystalloid solutions, but on the hydrophilic properties of the colloid and its retention in the circulating blood even under the conditions of increased vascular permeability prevailing in shock. Any osmotic effect which a colloid may exert, either directly or following its formation of salts, is of value only as long as the aforementioned prerequisites are fulfilled.

SUMMARY

Pectin solutions either freshly prepared and neutralized with phosphate buffer solution or autoclaved and neutralized were injected into dogs and rabbits.

The immediate effects produced on the blood by either of the two solutions are shown in colloidoclastic leukopenia, acceleration of erythrocytic sedimentation and moderate shortening of clotting time.

Repeated injections of pectin solutions do not cause any increase in the viscosity of the plasma or any changes in the number and the ratio of leukocytes.

In dogs and rabbits given injections of the freshly prepared pectin solution, marked foam-cellular storage phenomena develop in the spleen, liver, kidney and marrow in addition to foam-cellular atheromatosis of the various arteries. Older vascular lesions of this origin are characterized by hyaline intimal thickening with or without calcification and by hyaline necrosis and calcification in the media underneath.

Dogs and rabbits given injections of the autoclaved pectin solution showed, on the other hand, with the exception of 1 rabbit, only minor storage phenomena in the bone marrow and foci of hyaline degeneration and thickening of the arteries.

These differences in degree and type of reaction are due to the fact that pectin solutions are markedly depolymerized when exposed to heat and lose thereby much of their macromolecular characteristics and their original physicochemical properties.

These observations confirm the concept of the presence of a fundamental causal relation between physicochemical colloidal disturbances of the plasma and vascular atheromatosis and organic thesaurosis.

Case Reports

RECKLINGHAUSEN'S DISEASE WITH UNUSUAL SYMPTOMS FROM INTESTINAL NEUROFIBROMA

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A case of Recklinghausen's disease (general neurofibromatosis) is presented because of the unusual location of a particular tumor and its symptoms. A large tumor of the ileum was the origin of severe intermittent gastrointestinal bleeding in a woman 60 years of age, with an interesting history.

The patient was a German woman, single, 60 years old. The grandparents' history is not known. The father died at 81 of pneumonia. The mother was afflicted with general peripheral neurofibromatosis and died of "stomach cancer."

Cutaneous tumors developed in the 5 siblings of the patient at approximately the age of 40 years. At that age the tumors became more numerous and grew rapidly. In 1 sister obstruction of the bowel developed at 40, and a loop of gangrenous bowel was resected; she died postoperatively. No microscopic diagnosis is available in that case. A second sister died at 54 years of age with a "cancer of the spine." A third sister is about 54 years old at the time of writing. She has cutaneous tumors but no other findings. This sister is the only one of the children to have married. Her two sons, aged 24 and 26 years, do not show any signs of Recklinghausen's disease. The fourth sister is 46 years old. In addition to the neurofibromatosis of the skin, she has uterine fibroids, which occasionally have caused bleeding.

Finally, a brother aged 50 years has significant findings in addition to the cutaneous tumors. There are outstanding congenital defects about the face, and the right zygoma, though present, is not as prominent as the left. The right eyeball is small, and only the cornea is visible. This eye has perception of light only. The musculature of the right side of the face and the orbital fat are extremely atrophic. Both the upper and the lower jaw on this side are small, and no molar teeth ever appeared here.

The patient attended school for one year but never learned to read or write. She was accustomed to remain at home at all times and never married. At 20 years of age she was considered anemic. The tumors of the skin became prominent and numerous at about the age of 40. Shortly after her fiftieth birthday she noticed exertional dyspnea, which progressed until, two or three years later, definite heart failure set in. At the age of 59 she noticed, for the first time, gastrointestinal bleeding. At about the same time the right upper extremity became progressively weaker and atrophic, and finally deformity of the hand developed. Subsequently the hemorrhages became more frequent and severe each time, leaving the patient weak and pale. Lately the intestinal bleeding occurred about every four weeks, each time lasting from four to five days. The last episode of bleeding occurred in the last week of March 1941, and was so severe that the patient became almost exsanguinated and lost consciousness.

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The examination revealed an elderly white woman, pale, who was unable to walk because of weakness and who showed peripheral signs of Recklinghausen's disease. The temperature ranged from 100 to 102 F., and the pulse was consistently above 110 F. The heart was fibrillating, and the patient was dyspneic and orthopneic. There were basilar rales in the lungs. In the lower part of the abdomen was a round smooth mass, movable and painless. Digital rectal examination revealed a reddish black stool and external hemorrhoids. Proctoscopic examination and pelvic examination gave no unusual findings. At this time the blood count was: red blood cells, 2,840,000; white blood cells, 9,600; segmented forms, 55 per cent; stab forms, 38 per cent. The hemoglobin content was 39 per cent; the color index, 0.69.

The patient received three transfusions in addition to supportive treatment but failed to respond and died on the fifth hospital day with the complications of hypostatic pneumonia and heart failure.

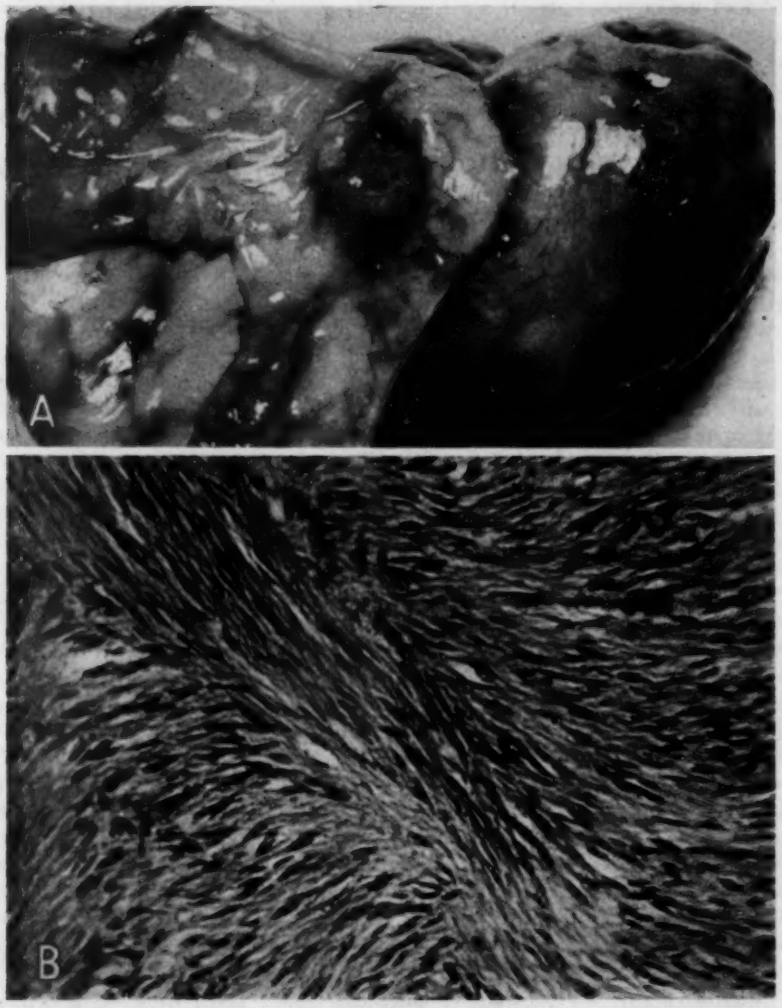
At autopsy the gross findings were interesting. The entire body, including the face, revealed numerous soft cutaneous tumors. Most of these were flat and averaged about 1 cm. in diameter. The largest measured 3 cm. in diameter and was located on the plantar aspect of the left foot. Some of these tumors were pedunculated, and at the base there could be felt a definite "hernial ring," described by dermatologists as characteristic of this disorder. The trunk and upper extremities showed in addition numerous brown pigmented spots. From the area of the right flank there arose an apron of dusky colored skin, measuring 16 by 12 cm. The right upper extremity was considerably smaller than the left. The musculature was atrophic, and the joints appeared to be ankylosed. The wrist was flexed, and the thumb was adducted. The most noticeable deformity was in the fingers. The proximal two phalanges were extended, while the distal was flexed, and all the interphalangeal joints were firmly ankylosed.

Pulmonary edema, congestion and hypostatic bronchopneumonia were present. The myocardium showed advanced brown atrophy. The liver was brownish, swollen and much softened. The spleen and kidneys were normal except for moderate hydronephrosis on the right side due to ureteral obstruction by an inflammatory reaction at the pelvic portion. The lumbar cord and the cauda equina showed no gross change. About 18 cm. proximal to the ileocecal valve there was a rounded tumor of the ileum. This was on the antimesenteric border and measured 6.5 by 5.5 by 4 cm. The surface of the tumor was smooth and glistening, and large distended blood vessels coursed over it (fig., *A*). The consistency was moderately soft, yet on sustained pressure it was firm. On hemisection the surface was homogeneous, moist, glistening and pinkish gray. Throughout there could be seen numerous, various-sized blood spaces. The majority of these ranged from pinhead to pea size. Some came directly in contact with the mucosa of the bowel, which was invaginated into the tumor mass for a distance of about 1.5 cm.

Microscopically, a representative cutaneous nodule taken from the abdominal wall and stained with hematoxylin-eosin showed a dense uniform reddish-staining collagen matrix containing elongated oval or pointed nuclei with compact granular bluish-staining chromatin substance. These nuclei were arranged haphazardly in all planes and directions and were uniform in size. There was no tendency toward palisade formation in the sections of the cutaneous tumor. The surface epithelium showed considerable atrophy, and close beneath the skin the tumor substance was separated by a heavy strand of rather acellular fibrous tissue. With the Masson[1] trichrome stain the matrix appeared as a greenish-staining

1. Masson, P.: J. Tech. Methods **12**:75, 1929.

substance with the nuclei scattered throughout the sections like those seen in the hematoxylin-eosin preparation. No definite whorls or bundle tracts could be made out, and no nerve filaments were found. A microscopic diagnosis of fibroma was made.



A, tumor of the ileum. Note the glistening surface and the large vessels coursing over it. The mucosa of the bowel can be seen at the base of the tumor, where it is invaginated into the tumor. *B*, high power photomicrograph showing the bundle tracts of wavy collagen with the parallel elongated dark granular nuclei.

The visceral tumor arose within the intestinal wall, and then, as it grew, became subserosal and carried with it a small pouch of intestinal epithelium, involving in this way practically all the layers of the intestinal wall except the mucosal surface. In many areas of the section one could find definite discon-

tinuity of the muscle layers, which were displaced when the tumor tissue split the smooth muscle fibers. The tumor came in direct contact with the submucosa, but this was not invaded. With hematoxylin-eosin stain the stroma of the tumor was made up of distinct bundles of reddish-staining tissue forming whorls and definite tracts, which in some places could be seen to shift their direction into other planes. These bundles showed wavy collagen fibers and elongated granular bluish-staining nuclei which lay parallel to one another (fig., *B*). Occasionally it was noticed that the nuclei arranged themselves parallel around a circumscribed area of collagen matrix giving in this way an impression of palisading. Careful scrutiny also showed the presence of large, practically oval vesicular nuclei with a scant amount of cytoplasm. These were interpreted as Schwann cells. One of the outstanding features of the tumor was the large number of thin-walled blood spaces. These were scattered throughout the entire tumor but were particularly abundant in the area nearest the epithelium of the bowel. Some of these blood spaces came in direct contact with the intestinal epithelium, and on following the epithelium one observed areas of erosion and necrosis. At such places the blood sinuses could be seen to open directly into the intestinal lumen. There were also some other irregularly outlined foci containing a pink-staining homogeneous material, apparently representing the edematous portion of the tumor as seen grossly.

With Masson's trichrome stain the intestinal tumor showed a relatively loose, wavy, green-staining collagen matrix, and in places there was a tinge of purple to the connective tissue. The intensity of the green staining was not uniform throughout all sections. In some places it was found to be rather dense and homogeneous, while in others it was found to be loose and wavy.

The Van Gieson stain showed fine wavy fibers staining red. The red-stained fibers were not uniform in their distribution and appeared similar morphologically to those found with the Masson trichrome stain.

Silver impregnation showed fine reticulin fibers parallel to the long axis of the nuclei in the bundle tracts, which on cross section formed a honeycomb pattern about some of the fibers in the tracts.

With the Smith-Quigley method for myelin sheaths it was impossible to demonstrate any myelinated nerve fibers in this tumor. In none of the sections were ganglion cells found.

The histologic picture of this tumor could very well be classified as type A tissue of Antoni[2]; i. e., it was rather uniform and made up of definite bundle tracts with parallel nuclei. In contrast, type B tissue is much more irregular in pattern and shows areas of distortion and myxomatous degeneration.

COMMENT

Origin of Tumors.—In the latter part of the nineteenth and the early part of the twentieth century there were in the French and German literature numerous reports dealing with the disorder which Recklinghausen[3] described in 1882. A short time later there appeared American articles in large numbers. The great majority of the authors wrote extensively on the nature and the origin of the tumors of Reckling-

2. Antoni, N. R. E.: Ueber Rückenmarkstumoren und Neurofibrome, Munich, J. F. Bergmann, 1920.

3. von Recklinghausen, F.: Ueber die multiplen Fibrome der Haut und ihre Beziehung zu den multiplen Neuromen, Festschrift zur Feier des fünfundzwanzigjährigen Bestehens des pathologischen Instituts zu Berlin; Herrn Rudolph Virchow dargebracht, Berlin, A. Hirschwald, 1882.

hausen's disease and allied tumors arising from central and peripheral nerves or their nerve sheaths. It is not in the scope of this paper to attempt to settle the ever present question, namely: From what do these tumors originate? Opinion is about equally divided. One school believes that such tumors originate from the perineurium or endoneurium and hence are mesodermal. This opinion is shared by Antoni,[2] Bailey and Herrmann,[4] Gray,[5] Harbitz,[6] Hosoi,[7] Penfield and Young [8] and Stout.[9] The other school traces the same tumors to proliferation of nerve sheath cells or the cells of Schwann. However, both generally agree that Schwann cells are ectodermal in origin. This has been proved by Harrison.[10] In agreement with the ectodermal derivation of such tumors is a long list of writers, headed by Masson [11] among the recent ones. Others in this school are Cushing and Eisendrath,[12] Foot,[13] Geschickter,[14] Ghyselen,[15] Korbsch,[16] Stewart and Copeland [17] and Verocay.[18] Lewis and Hart [19] took a stand midway between these opinions and stated that both elements take part in the formation of these tumors. This opinion is shared by us after the study of this and similar cases.

Histologic Character.—Extensive cytologic studies of the tumors of Recklinghausen's disease have been reported by American authors and prolifically by the Germans. Some describe nerve fibers and ganglion cells in these tumors. However, basically the cytologic picture of the intestinal tumor in the case reported here fits in readily with those of the tumors of Recklinghausen's disease described by Antoni,[2] Genersich,[20] Herxheimer and Roth,[21] Penfield,[22] Stout,[9] Verocay [18] and Recklinghausen. Hosoi [7] laid emphasis on cancerous changes in the tumors of

4. Bailey, P., and Herrmann, J. D.: Am. J. Path. **14**:1, 1938.

5. Gray, S. H.: Arch. Neurol. & Psychiat. **22**:98, 1929.

6. Harbitz, F.: Arch. Int. Med. **3**:32, 1909.

7. Hosoi, K.: Arch. Surg. **22**:258, 1931.

8. Penfield, W., and Young, A. W.: Arch. Neurol. & Psychiat. **23**:320, 1930.

9. Stout, A. P., and Carson, W.: Am. J. Cancer **24**:751, 1935; cited by Stout.[59]

10. Harrison, R. G.: J. Comp. Neurol. **37**:123, 1924.

11. Masson, P.: Am. J. Path. **8**:367, 1932.

12. Cushing, H., and Eisendrath, L.: Meningiomas: Their Classification, Regional Behavior, Life History, and Surgical End-Results, Springfield, Ill., Charles C. Thomas, Publisher, 1938.

13. Foot, N. C.: Am. J. Clin. Path. **6**:1, 1936.

14. Geschickter, C. F.: Am. J. Cancer **25**:377, 1935.

15. Ghyselen, R.: Rev. belge sc. méd. **6**:519, 1934.

16. Korbsch, H.: Arch. f. Psychiat. **92**:183, 1930; abstracted, Am. J. Cancer **15**:1869, 1931.

17. Stewart, F. W., and Copeland, M.: Am. J. Cancer **15**:1235, 1931.

18. Verocay, J.: Beitr. z. path. Anat. u. z. allg. Path. **48**:1, 1910.

19. Lewis, D., and Hart, D.: Ann. Surg. **92**:961, 1930.

20. Genersich, A.: Virchows Arch. f. path. Anat. **49**:15, 1870.

21. Herxheimer, G., and Roth, W.: Beitr. z. path. Anat. u. z. allg. Path. **58**: 319, 1914.

22. Penfield, W.: Tumors of the Sheaths of the Nervous System, in Cytology and Cellular Pathology of the Nervous System, New York, Paul B. Hoeber, Inc., 1932, vol. 3, p. 953.

Recklinghausen's disease and gave the figure as 13 per cent. We observed such changes in a patient who came to autopsy. A 30 year old woman with general neurofibromatosis presented sarcomatous change in an axillary tumor. There was no metastasis, but the local growth was large.

Incidence of Intestinal Tumors.—The rarity of benign intestinal tumors in this disease becomes evident when one considers a few statistical reports on the subject. In a special study of benign fibroma of the intestine King [23] found myoma of the jejunum in 1 of 44,644 intraperitoneal operations. At that time he found in the literature only 3 cases of neurofibroma of the intestine among 119 authentic cases of noncancerous intestinal tumor. Raiford,[24] from 11,500 autopsies and 45,000 surgical specimens at Johns Hopkins Hospital, was able to collect but 85 cases of intestinal tumor and in none of these was the tumor associated with Recklinghausen's disease. From the literature he collected 339 cases of tumor of the small intestine. Geschickter [25] collected 178 cases of noncancerous tumor of the digestive tract. In this series he listed 10 cases under the title "Lipoma, Fibroma, Neuroma," but did not elaborate further. Stout [9] in his report on neurilemmoma cited 40 articles in which the gastrointestinal tract was discussed. In the majority of the cases the tumor occupied the stomach and duodenum and was not associated with Recklinghausen's disease.

An attempt was made by us to study the literature on the subject of tumors of the small intestine of nervous origin, particularly in their association with Recklinghausen's disease. In this study we found 16 cases of intestinal neurofibroma. Of these, only 5 presented symptoms referable to some pathologic lesion of the intestinal tract. Such cases have been reported by Askanazy,[26] Banerjee and Christeller [27] (case 2), Leriche,[28] Schneider,[29] and Wolff.[30] In the remaining 11 cases there were no clinical symptoms whatever, and the intestinal findings were incidental, usually encountered at autopsy. These are the cases mentioned by Adrian,[31] Banerjee and Christeller [27] (case 1), Branca,[32] Conos and Archélaos,[33] Genersich,[20] Hansemann,[34] Heine,[35] Kohtz,[36] Marie,[37]

23. King, E. L.: Surg., Gynec. & Obst. **25**:54, 1917.

24. Raiford, T. S.: Arch. Surg. **25**:122, 1932.

25. Geschickter, C. F.: Am. J. Cancer **25**:130, 1935.

26. Askanazy: Arb. a. d. path. Inst. Tübingen **2**:3, 1899; abstracted, Centralbl. f. allg. Path. u. path. Anat. **2**:54, 1900.

27. Banerjee, D. N., and Christeller, E.: Virchows Arch. f. path. Anat. **261**: 50, 1926.

28. Leriche, R.: Lyon chir. **6**:70, 1911.

29. Schneider: Zentralbl. f. Chir. **59**:2245, 1932.

30. Wolff, P.: Schweiz. med. Wchnschr. **66**:379, 1936.

31. Adrian, C.: Wien. klin. Wchnschr. **15**:813, 1902.

32. Branca, A.: Compt. rend. Soc. de biol. **48**:1124, 1896.

33. Conos, B., and Archélaos, S.: Rev. neurol. **38**:78, 1931; abstracted, Am. J. Cancer **15**:1870, 1931.

34. Hansemann: Berl. klin. Wchnschr. **32**:662, 1895.

35. Heine, J.: München. med. Wchnschr. **74**:259, 1927.

36. Kohtz, H.: Ein Fall von multiplen Fibromen der Haut, Inaug. Dissert., Königsberg, 1893, p. 28; cited by Hosoi.[7]

37. Marie, F., and Couvelaire: Nouv. iconog. de la Salpêtrière **13**:26, 1900; cited by King.[23]

Preble and Hektoen [38] and Recklinghausen.[3] Neurinomas of the small intestine which were not associated with Recklinghausen's disease but which produced symptoms have been recorded by Berggrun,[39] Diez,[40] Dudley,[41] Guberman,[42] König,[43] Lemonnier and Peycelon,[44] Nordlander,[45] and Serafini.[46] Very similar are the cases of Modrezewski,[47] Krieleis [39] and Tauber.[48] The diagnosis in these cases was given as myoma or fibromyoma, but the meagerness of the microscopic descriptions leads one to doubt the interpretations. A case of cancerous neuroma of the ileum causing peritonitis was reported by Bergendal and Sjövall.[49] In their microscopic description they pointed out frequent mitotic figures. The patient died three years later with general abdominal metastasis.

The symptoms produced by intestinal tumors of Recklinghausen's disease are variable and never in any degree characteristic of the disorder. They consist usually of vague abdominal discomfort, bleeding and intestinal obstruction. Raiford,[24] Rhodenberg [50] and Dewes [51] discussed the symptoms of such tumors more fully. Jones and Hart [52] discussed surgical complications of neurofibromatosis but did not mention intestinal bleeding.

Associated Findings.—Of the sundry combinations which are frequently associated with cutaneous neurofibromatosis, this particular case presents three in addition to the intestinal tumor. These include hereditary history, low mentality and arthritis deformans. Stewart and Copeland [17] cited some twenty or more associated findings, which range from endocrinologic disturbances to changes involving the central nervous system, the vascular system and the osseous system. Reuben [53] and Le Bell,[54] in reporting their cases of Recklinghausen's disease in children also mentioned the variety of pathologic conditions which frequently are found associated with the disease.

Heredity.—The presence of the disease in the mother and the siblings of this patient brings us to study its hereditary tendency. The

38. Preble, R. B., and Hektoen, L.: Am. J. M. Sc. **121**:1, 1901.

39. Cited by Herxheimer and Roth.[21]

40. Rivas Díez, B.: Semana méd. **44**:457, 1937.

41. Dudley, G. S.: S. Clin. North America **10**:539, 1930.

42. Guberman, M. O., in Melnikoff-Razvedenkoff: Patologoanatomicheskaya kazuïstika, Moscow, A. A. Levinson, 1904, p. 68; abstracted, Ergebn. d. allg. Path. u. path. Anat. **10**:14, 1904-1905.

43. König, E.: Chirurg **4**:636, 1932; abstracted, Zentralbl. f. Chir. **60**:526, 1933.

44. Lemonnier and Peycelon: Bull. et mém. Soc. nat. de chir. **59**:1318, 1933.

45. Nordlander, E.: Upsala läkeref. förh. (art. XVIII), 1932, vol. 38.

46. Serafini, G.: Cancro **2**:281, 1931; abstracted, Cancer Rev. **7**:409, 1932.

47. Modrezewski, E.: Berl. klin. Wchnschr. **19**:627, 1882.

48. Tauber, R.: Wien. klin. Wchnschr. **36**:780, 1923.

49. Bergendal, S., and Sjövall, A.: Chirurg **9**:573, 1937; abstracted, Am. J. Cancer **37**:478, 1939.

50. Rhodenberg, G. L.: J. Lab. & Clin. Med. **4**:434, 1919.

51. Dewes, J.: Boston M. & S. J. **155**:427, 1906.

52. Jones, R., and Hart, D.: Ann. Surg. **110**:916, 1939.

53. Reuben, M.: Arch. Pediat. **51**:522, 1934.

54. Le Bell, I. C.: Arch. Pediat. **54**:454, 1937.

report of Gardner and Frazier [55] is outstanding in this respect. These authors reported 38 members in five generations of one family who were afflicted with bilateral acoustic nerve tumors. Preiser and Davenport [56] also commented on the hereditary nature of Recklinghausen's neurofibromatosis, concluding that it was a dominant character. In several of the cases reported by various authors there was also an associated familial history—for example the cases reported by Bailey and Herrmann,[4] Harbitz,[6] Herxheimer and Roth [21] and Adrian.[51] Hoekstra [57] stated that the congenital inclination to the disorder of neurofibromatosis occurred in four generations and increased through these. In a study of twins Siemens,[58] on the other hand, came to the conclusion that it was not a simple dominant hereditary disease. The symptoms could develop in the various members of one family, but, he stated, of uniovular twins, one may show the disease and not the other.

Mentality.—It was observed by the older writers that some patients with neurofibromatosis were low in mentality. In case 7 of Harbitz [6] the patient bordered on imbecility. Stout [59] and Adrian [60] each cited a case of Recklinghausen's disease in which there was some degree of mental deficiency. Preiser and Davenport [56] gave the incidence of feeblemindedness in patients with Recklinghausen's disease as 7.8 per cent, or twenty times that in the average population. Our patient is described as an illiterate, backward introvert.

Finally this patient showed arthritis deformans of the right upper extremity. Similar involvement of the osseous system has been reported by other authors. The case of Preble and Hektoen [38] was one in which there was demonstrated extensive deforming arthritis of the spine and extremities in a 30 year old woman, who also had scattered irregular nodular patches of the jejunum. Adrian's [51] patient with cutaneous and intestinal neurofibroma also had arthritis deformans of the right upper extremity. The patient in Adrian's [60a] case 5 had kyphoscoliosis. Miller and Frank [61] and Moore [62] discussed further the bony findings which may be associated with Recklinghausen's disease. Lehman [63] gave three groups of bony changes: scoliosis, overgrowth of individual bones and irregularity of the contour of bones.

Our patient's brother, with facial asymmetry and maldevelopment of the orbit, its contents and the jaws, presents a picture similar to that described previously by others. The predilection for the craniofacial bones is reported by Steinsleger and Slullitel,[64] Weber and Bode,[65] Uhlmann and Grossman,[66] and Heine.[35]

55. Gardner, W. J., and Frazier, C. H.: Arch. Neurol. & Psychiat. **23**:226, 1930.

56. Preiser, S. A., and Davenport, C. B.: Am. J. M. Sc. **156**:507, 1918.

57. Hoekstra, C.: Virchows Arch. f. path. Anat. **237**:79, 1922.

58. Siemens, H. W.: Virchows Arch. f. path. Anat. **260**:234, 1926.

59. Stout, A. P.: Am. J. Cancer **25**:1, 1935.

60. Adrian, C.: (*a*) Beitr. z. klin. Chir. **31**:1, 1901; (*b*) footnote 31.

61. Miller, A. J., and Frank, L. W.: Ann. Surg. **109**:246, 1939.

62. Moore, B. H.: J. Bone & Joint Surg. **23**:109, 1941.

63. Lehman, E.: Arch. Dermat. & Syph. **14**:178, 1926.

64. Steinsleger, M., and Slullitel, I.: Semana méd. **1**:481, 1934; abstracted, Am. J. Cancer **22**:971, 1934.

65. Weber, F. P., and Bode, O. B.: Proc. Roy. Soc. Med. **27**:638, 1934.

66. Uhlmann, E., and Grossman, A.: Ann. Int. Med. **14**:225, 1940.

SUMMARY

It is evident from a study of the literature that noncancerous tumor of the small intestine is quite uncommon and, furthermore, that it is extremely rare to find a patient with neurofibroma of the small bowel (exclusive of the duodenum) presenting serious clinical symptoms. The case now reported is that of a white woman who had peripheral cutaneous neurofibroma of Recklinghausen's disease and associated abnormalities. The chief complaint concerned massive intermittent gastrointestinal bleeding from a very large vascular tumor of the ileum, diagnosed as neurofibroma. The patient also presented three other interesting conditions, namely, a familial-hereditary tendency, low mentality and arthritis deformans.

PAPILLOMA OF THE CHOROID PLEXUS

Report of a Case and Summary of Recorded Cases

LOUIS C. POSEY, M.D., BIRMINGHAM, ALA.

Among cerebral neoplasm papilloma of the choroid plexus is unusual. Cushing [1] found only 12 cases of this neoplasm among 2,023 verified cases of intracranial tumor. Three excellent summaries of the literature [2] are available. Since the last review [2c] 9 additional cases have been reported.[3] The instance reported by Hart [4] apparently was not included in these reviews. The present case brings the total number of recorded cases of papilloma of the choroid plexus to 86.

Only twice has papilloma of the choroid plexus of the third ventricle been reported in a child under 2 years of age.[5] Therefore, because of its rarity, the case presented here seems worthy of record.

REPORT OF CASE

A 6 month old white girl was admitted to the Children's Hospital (service of Dr. Hughes Kennedy) Dec. 11, 1939 in a stupor. She had been vomiting and for a period of two weeks had had "a cold." Four days before admission marked pallor was noted. On the day before, the left ear drum had been opened. No fever had been present, and on admission the temperature was 98.6 F. The baby appeared chronically ill and had a little drainage in the left external auditory canal. The anterior fontanel was soft and not bulging. There was questionable spasticity of the right leg. Occasional extrasystoles were noted, and the edge of the liver was 1 fingerbreadth below the right costal margin. A small incision was present in the left ear drum.

The red blood cell count was 4,200,000, the hemoglobin content was 74 per cent of normal. The white blood cell count was 9,650, with 29 per cent lymphocytes, 7 per cent monocytes, 60 per cent neutrophils, 3 per cent eosinophils and 1 per cent basophils.

The morning after admission the infant appeared stuporous and vomited after taking the formula, orange juice or water. At noon, on spinal puncture the fluid

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1. Cushing, H.: Intracranial Tumors, Springfield, Ill., Charles C. Thomas, Publisher, 1932.

2. (*a*) Van Wagenen, W. P.: Arch. Surg. **20**:199, 1930. (*b*) Friedman, J. F., and Solomon, C. I.: Am. J. Dis. Child. **52**:114, 1936. (*c*) Turner, A. O., and Simon, M. A.: Am. J. Cancer **30**:289, 1937.

3. (*a*) Sai, S.: Taiwan Igakkai Zasshi **36**:1590, 1937. (*b*) Ebbs, J. H.: Arch. Dis. Childhood **12**:403, 1937. (*c*) Cohen, I.: J. Mt. Sinai Hosp. **4**:798, 1938. (*d*) Saccone, A., and Rosenthal, A.: Arch. Path. **25**:850, 1938. (*e*) Weinstein, E. A.: J. Mt. Sinai Hosp. **5**:573, 1938 (3 cases). (*f*) Drucker, G. A.: Arch. Path. **28**:390, 1939. (*g*) Caron, S., and Samson, M.: Laval méd. **4**:217, 1939.

4. Hart, K.: Arch. f. Psychiat. u. Nervenh. **47**:267, 1910.

5. Henneberg: Berl. klin. Wchnschr. **12**:277, 1903. Okabe, Y.: Gann **18**:28, 1924.

was clear and rushed out under increased pressure. While the child was still on the table, respiration ceased. A 25 per cent solution of pyridine betacarboxylic acid diethylamide (coramine) and artificial respiration were administered, and spontaneous breathing returned. The stupor deepened. Fluids and the formula were refused. A second spinal tap was made during the night after admission. The fluid was clear and the pressure high. Respiration became slow, the pulse weakened and during the twenty-fifth hour after admission the baby died.

The positive findings on postmortem examination were confined to the cranium. The craniothoracic ratio was 1.10 (43/39 cm.). The dura and its sinuses were natural. The brain was voluminous. A slight conical deformity was present at the base of the cerebellum. This deformity continued around the medulla as a

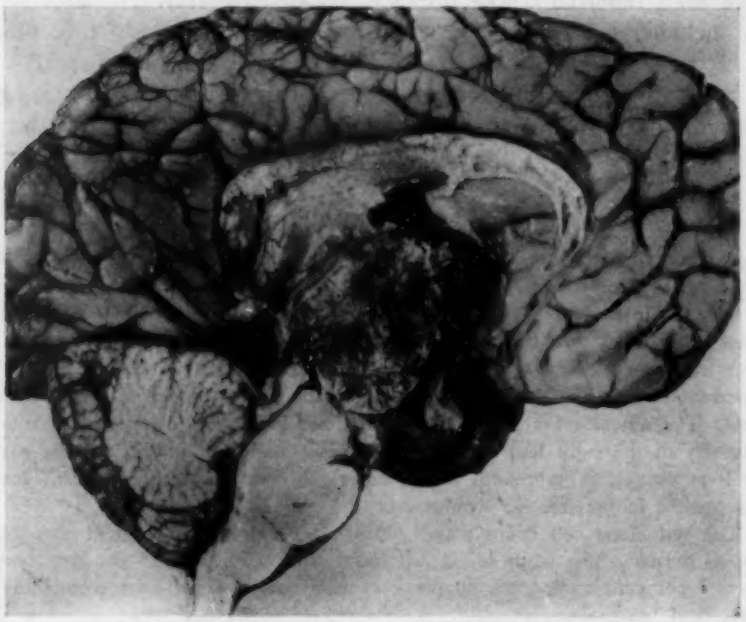

Fig. 1.—Midsagittal section of the brain showing the tumor mass filling the third ventricle and obstructing the cerebral aqueduct. The dilatation of the lateral ventricle can be seen through the opening in the septum.

shallow groove across the anterior surface. Cerebral vessels were compressed. The convolutions were broad and flattened. The third ventricle bulged downward and forward, pushing before it the optic tracts and the chiasm. The tracts were flattened greatly. When the pituitary stalk was severed, there was an escape of fluid from the third ventricle. The fluid was clear. Through the rent in the floor of the third ventricle could be seen a tumor mass that filled the greater part of the ventricle. In the midsagittal plane (fig. 1) the tumor was seen to be an encapsulated semifirm structure attached laterally and basally to the ependyma of the third ventricle. Posteriorly, the mass overlay the orifice of the cerebral aqueduct. The tumor measured 3.5 by 2.5 by 2.5 cm. The cut surface of the

tumor showed a finely divided villous-like structure of brittle consistency. The tumor was grayish pink. There was marked dilatation of the third ventricle and of both lateral ventricles. The aqueduct and the fourth ventricle were not dilated. No secondary tumor masses were present.

A serous exudate was observed in both middle ears, more on the left side.

Microscopically, the tumor showed branching connective tissue columns covered by tall columnar to cuboidal epithelium (fig. 2). The columns were richly supplied with small blood vessels. The epithelium was well differentiated, but there were occasional tertiary branches of epithelial cells only. Some of these epithelial masses were detached and resembled "placental giant cells." Except for abundancy of structure there was no difference in histologic appearance between this tumor and the normal choroid plexus of infancy. No blepharoplasts or cilia could be made out. The microscopic diagnosis was papilloma of the choroid plexus.

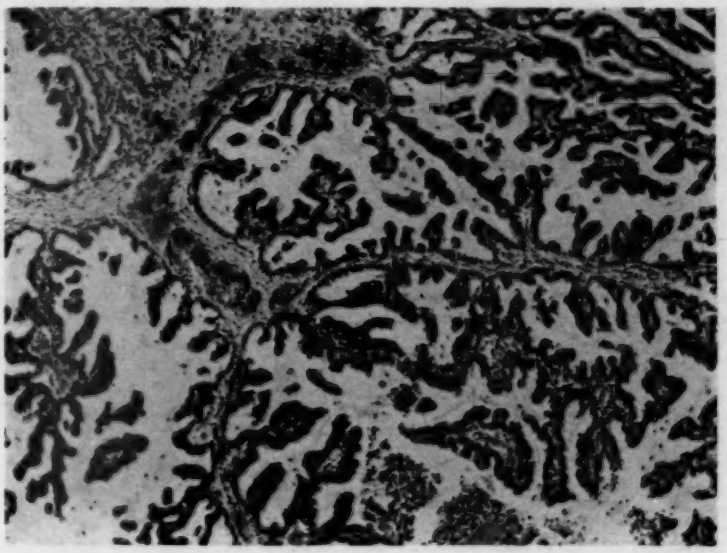

Fig. 2.—Microscopic section showing numerous papillary formations covered by low columnar cells; × 75. Note the large vascular sinuses in the connective tissue columns.

COMMENT

Papilloma of the choroid plexus was described for the first time in 1832 by Guérard.[6] The tumor he described occurred in the right lateral ventricle of a 3 year old girl. The tumor was encapsulated and about the size of a hen's egg. Tumors of this type have been observed with increasing frequency. During the past two decades 55 have been recorded.

The location of these tumors in the cerebral ventricles is a point of particular interest because of certain peculiarities. The lateral ventricles are the commonest sites of papilloma in infancy, while the fourth

6. Guérard: Bull. Soc. anat. de Paris 8:21, 1832.

ventricle is the most frequent site in adulthood. The left lateral ventricle is involved twice as commonly as the right lateral ventricle. The fourth ventricle is the principal site of papilloma of the choroid plexus. It should be pointed out that a tumor of the fourth ventricle is very apt to produce symptoms, and patients with this condition have been commonly subjected to surgical operations. In 1 instance both lateral ventricles were involved [7]. The spinal dura has been the primary site of papilloma, the tumor being of microscopic size [8]. Table 1 shows the location of the tumors reported to date.

The high incidence of papilloma of the choroid plexus in early life is shown in table 2. It is not uncommon during the first year of life.

TABLE 1.—*Location of Papilloma of the Choroid Plexus in Recorded Cases*

Cerebral Location	Cases
Left lateral ventricle	22
Right lateral ventricle	11
Both lateral ventricles	1
Third ventricle	13
Fourth ventricle	38
Spinal dura	1
	86

TABLE 2.—*Age Incidence of Papilloma of the Choroid Plexus in Recorded Cases*

Years of Age	Cases
0- 9	27
10-19	9
20-29	11
30-39	7
40-49	10
50-59	5
60-69	2
70-79	1
	72*

* The ages were not available in most of the remaining 14 cases.

It has occurred in a newborn infant.[3f] It has been observed in only a few persons of the "cancer age."

Hydrocephalus was observed in half the recorded cases. In patients of the lower age group hydrocephalus was almost always present, while in adults hydrocephalus was more often absent. In some cases hydrocephalus was due to obstruction to the flow of ventricular fluid, while in others claims have been made that the tumor produced excessive fluid.

There is a lack of information as to the sex incidence of papilloma of the choroid plexus. In 14 of the recorded cases the patients were females; in 25, males, and in 47 the sex was not recorded.

7. von Plath: Jahrb. f. Kinderh. **21**:417, 1884.

8. Hall, G. W., and Fentress, T. L.: J. Neurol. & Psychopath. **14**:108, 1933.

The tumors are described as papillary, villous, cystic or encapsulated masses. They are usually reddish gray. The masses vary in size from a microscopic structure to an enormous growth. The average diameter was 3 to 4 cm. As a rule, the lesions were solitary. Secondary implants have been described in various locations to which cells were transferred through the current of the cerebrospinal fluid. This process, called seeding, was present in 9 cases.[9] Histologic cancer was present in 2 cases.[2c] Clinical cancer was described in 4 cases.[10] Probable cancer was mentioned in 3 cases.[11] In the remaining 77 cases the tumor was described as noncancerous.

Papilloma of the choroid plexus reproduces the histologic characteristics of the choroid plexus of infancy. There are branching, vascularized connective tissue columns covered by tall columnar epithelium. In some instances the epithelium is ciliated. The cancerous lesions are anaplastic solid masses, or poorly defined sheets of cells, showing little tendency to papillary formation. Multilayering of the surface cells was common in the cancerous lesions.

TABLE 3.—*Hydrocephalus in Recorded Cases of Papilloma of the Choroid Plexus*

	Cases in Which			
Age Group	Hydrocephalus Was Present	Hydrocephalus Was Absent	Information on This Point Was Not Available	Total
Under 20 years	27	0	9	36
20 years or over	14	22	14	50
	41	22	23	86

The diagnosis of papilloma of the choroid plexus has been made usually at autopsy. In 22 cases it was diagnosed by surgical methods.[12]

9. (*a*) Le Blanc, C.: Beitrag zur pathologischen Anatomie der Gehirntumoren, Inaug. Dissert., Bonn, F. Krüger, 1868. (*b*) Unger, E., and Bielschowsky, M.: Arch. f. klin. Chir. **81**:61, 1902. (*c*) Hart.[4] (*d*) van Bouwdijk Bastiaanse, F. S.: Ztschr. f. d. ges. Neurol. u. Psychiat. **27**:96, 1914. (*e*) Toppich, G.: Frankfurt. Ztschr. f. Path. **33**:238, 1925. (*f*) Van Wagenen[2a] (case 2). (*g*) Kellner, B.: Virchows Arch. f. path. Anat. **289**:656, 1933. (*h*) Bleyer, A., and Siebert, W. J.: J. Pediat. **8**:193, 1936.

10. (*a*) Zdrahol, N.: Arch. f. Kinderh. **109**:24, 1936. (*b*) Faber, V.: Frankfurt. Ztschr. f. Path. **47**:168, 1934. (*c*) Dandy, W. E.: Benign Tumors in the Lateral Ventricle of the Brain, Baltimore, Williams & Wilkins Company, 1934 (2 cases).

11. Audry, J.: Rev. de méd. **11**:897, 1886. Unger and Bielschowsky.[9b] van Bouwdijk Bastiaanse.[9d]

12. (*a*) Unger and Bielschowsky.[9b] (*b*) van Bouwdijk Bastiaanse.[9d] (*c*) Perthes: München. med. Wchnschr. **66**:677, 1919. (*d*) Sachs, E.: Arch. Neurol. & Psychiat. **8**:379, 1922. (*e*) Davis, L. E., and Cushing, H.: ibid. **13**:681, 1925 (5 cases). (*f*) Van Wagenen[2a] (1 case). (*g*) Butterfield, D. L.: New York

(Footnote continued on next page)

Complete surgical recovery was recorded in 5 cases,[13] and partial recovery was obtained in 1 case.[12g] In 14 of the surgical cases the tumor was located in the fourth ventricle; in 5, in the left lateral ventricle; in 1, in the right lateral ventricle, and in 1, in the third ventricle.

The principal stumbling block in the diagnosis of these tumors no doubt is the lack of symptoms due to the location of the papilloma. Hydrocephalus should not be overlooked in infancy and in early childhood. Careful craniothoracic measurements may assist in the detection of enlargement of the head. In the absence of other symptoms, ventriculography offers a method of localization.[14] The tumors may reach considerable size before symptoms appear. In the case reported here enlargement of the head was so slight as to be unnoticed until autopsy.

SUMMARY

A case of papilloma of the choroid plexus of the third ventricle in a 6 month old white girl is reported. A short summary of recorded cases is given.

State J. Med. **31**:1007, 1931. (*h*) Guillain, G.; Petit-Dutaillis, D.; Bertrand, I., and Lereboullet, J.: Rev. neurol. **39**:497, 1932. (*i*) Cushing,[1] p. 129 (6 cases). (*j*) Dandy, W. E.: Benign Tumors in Third Ventricle of Brain, Springfield, Ill., Charles C. Thomas, Publisher, 1933, p. 83. (*k*) Dandy[10e] (1 case). (*l*) Obarrio, J. M.; Dowling, E., and Pedace, E. A.: Semana méd. **1**:2, 1934. (*m*) Cohen.[3c]

13. Perthes.[12c] Sachs.[12d] Van Wagenen[2a] (case 1). Obarrio and others.[12l] Cohen.[3c]

14. Dandy, W. E.: Arch. Surg. **98**:841, 1933.

General Reviews

EFFECTS OF RADIATION ON NORMAL TISSUES

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V. EFFECTS ON THE RESPIRATORY SYSTEM

The lung has been more carefully studied with respect to its response to radiant energy [1] than other parenchymatous organs. This is due largely to the frequent use of radiation in the treatment of carcinoma of the breast and consequent exposure of the lungs. But radiation pneumonitis (or pleuropneumonitis as described by the roentgenologists) was recognized as a complication of radiation treatment of the thoracic wall only as the technics providing greater depth of dosage came into general use (Groover and others, 1927). It then became clear that the total radiant energy reaching the lung in a given period, rather than the quality of the rays, determined the degree of injury (Evans and Leucutia; Wintz, 1923 b).

There are two principal sources of knowledge of changes in the lung induced by radiation: first, postmortem anatomic observations correlated with the clinical and roentgenologic changes; second, experimental observations.

GENERAL CLINICAL OBSERVATIONS

It is now generally recognized that inflammation of the lung frequently, but not inevitably, follows therapeutic irradiation of the chest (Desjardins; Kaplan and Bell). This reaction is evident from the cough, dyspnea, occasionally fever, sometimes pain and pleural effusion which usually start two to three weeks after treatment.[2] From the onset of these symptoms of respiratory embarrassment, one can follow a series of changes in the roentgenograms which are quite typical of radiation pneumonitis. At first there is unusual radiolucency (Warren and Spencer), followed in days or weeks by increased density which starts in the hilus and spreads until, in the course of three or four weeks, it involves practically the entire lung. Later, this density may become bandlike or mottled (Davis). The mediastinal shadow is displaced toward the affected side, and fixation, elevation of the diaphragm and restriction of the expansion of this side may be present (Desjardins;

1. That is, radiant energy from roentgen radiation, radon, radium and temporarily radioactive compounds used therapeutically.

2. Desjardins. Hsieh and Kimm. Groover and others, 1927.

Groover and others, 1927). The parenchymal shadows suggest pneumonia or influenza (Groover and others, 1922) but are more often mistaken for metastatic tumor (Ruggles). This error may be disastrous for the patient if it leads to further irradiation of the already injured lung (Freid and Goldberg). Prolonged observation is often necessary before a diagnosis of radiation reaction of the lung can be made (Engelstad, 1937); the general condition of the patient is usually much better than one would expect from the symptoms and the extent of the lesion, were it any other process. In the majority of cases there is slow regression after the maximum reaction is reached, and the lung eventually, in the course of weeks or months, assumes the normal appearance (Wintz, 1923 a). Residual fibrosis is usually found only after heavy doses or long-continued or ill timed exposure. Fatalities do occur and are nearly always the result of ignorance of the reaction of the lung to radiation and its consequent vulnerability to further radiation and to infection (Warren and Spencer; Wintz, 1923 a).

The conditions which influence the reaction are not all understood. The presence or the degree of reaction is not predictable from the amount of radiation which is given (Davis). Signs and symptoms are usually first apparent after the third and subsequent courses of treatment and are closely related to erythema of the skin (Groover and others, 1923), but the reaction may be apparent after the first series and without erythema (Groover and others, 1927). It is thought that even in the absence of objective signs of injury the resistance of the lung may be lowered by a single suberythema dose (50 to 60 per cent of an erythema dose) (Wintz, 1923 b) or by even smaller doses (30 to 40 per cent) repeated at short intervals over a long period (Evans and Leucutia).

Many factors may be responsible for differences of susceptibility: age (McIntosh and Spitz), thickness of the wall of the chest (Groover and others, 1927), the presence or absence of pathologic conditions in the lung at the time of exposure to radiation (Ewing) and conditions favoring atelectasis (Groover and others, 1927). However, the relative importance of all of these is largely a matter of assumption (Warren and Spencer).

The only report of a large series of autopsies (234) on patients who had received radiation to the thorax gives the incidence of histologic evidence of injury from roentgen rays as 12 per cent (Warren and Spencer). The characteristic changes will be described in detail farther on.

GENERAL EXPERIMENTAL OBSERVATIONS

There has been much experimental work on the effect of radiation on the normal lung. Early studies dealt largely with the effect of radiation as used in the treatment of experimental pulmonary tuberculosis.

Neglect in considering the dual role of infection and radiation led to certain misconceptions. Thus, pleural fibrosis was stressed as an important result of exposure to radiation (Bergonié and Teissier). Some workers (Küpferle and Bacmeister, 1913; Küpferle) attributed increased fibrosis and calcification of active tuberculous lesions to radiation, but only when this was given in large amounts; the relation was not found by others (Gorke and Töppich). This early emphasis on fibrosis as a radiation effect probably unduly influenced roentgenologists in rationalizing the roentgenographic effects seen after the use of radiation. Acute bronchitis and pneumonic manifestations were described as the result of radiation injury in the nontuberculous portions of the lung (Küpferle and Bacmeister, 1916).

Recent experimental work has shown a close parallel between the changes induced by radiation in man and in animals. The same symptoms, roentgenologic aspects and variations in susceptibility among persons have been noticed within animal species. Attempts to determine threshold doses for the different types of reactions have been only partially successful.

The extensive studies of Engelstad (1934 a) have done much to establish knowledge of the experimental condition, and the reader is referred to his monograph for a detailed and critical summary of the experimental work. The correlation of the observations in man and in animals by Warren and Gates has further clarified understanding of the pathologic process.

ANATOMIC CHANGES IN MAN AND IN ANIMALS

The changes in the lung induced by radiation as seen in animals (rabbits, guinea pigs, dogs, rats, shoats) and in man will be discussed in relation to the various histologic units.

Parenchyma.—(*a*) Early Minor Reaction: The first effect of radiation on the animal lung appears hours or days after exposure and is a transient one, similar to erythema of the skin. Dilatation and engorgement of capillaries have been described as occurring in a matter of hours after exposure to radiation, whether the doses are moderate or heavy,[3] in a variety of laboratory animals,[4] varying in intensity with the dose

3. Such rough approximations of the specific doses as "moderate," "light," "heavy" without reference to voltage, filter, distance, aperature and time may seem too gross, but the data and conditions are so diverse that one must choose between a minute report, which a review of this type dos not permit, and a rough summing up. Fortunately, the latitude of change is so great that from the histologic point of view more details would be almost impertinent.

4. Lazarus-Barlow. Davis. Luden and Werthemann. Flaskamp. Granzow. Wohlauer. Warren and Gates.

(Tsuzuki). The duration of reaction depends on the dose and on the period during which it is given. In rabbits, after moderate doses the reaction disappears within a week. Engelstad's (1934 a) observations on rabbits one and two months after 7,040 r had been given in three doses two days apart indicate that hyperemia either persists for a long time or recurs. Petechial hemorrhages may be present with hyperemia, or they may be the only changes seen in the lungs after heavy doses of radiation, causing death of guinea pigs in twenty-four to forty-eight hours (Wohlauer; Granzow).

Edema is often, though not always, coincident with congestion, but it is usually seen early (Davis; Lucarelli). Following heavy or repeated light doses, the edema may be extreme, increasing the bulk of the connective tissue and distending the alveoli.[5]

Lymphectasia, though not often described, is probably a fairly common reaction (Warren and Gates; Wohlauer).

A slight cellular infiltration sometimes appears along with the vascular response. It is hardly appreciable except after heavy doses (Engelstad, 1934 a; Warren and Gates). Whereas Lucarelli found congestion and edema in rabbits twenty-four hours after three doses of 50 per cent of an erythema dose two days apart, he described cell response only after five doses of 50 per cent of an erythema dose two days apart. This response consisted of the presence of eosinophils (which correspond to neutrophils in man) in the interalveolar septums twenty-four hours after the last exposure to radiation and two weeks later; but Engelstad (1934 a) described infiltration of peribronchial and perivascular tissues by cells four hours after a single dose of 1,700 r, lasting from three to twenty days.

Patchy, irregular atelectasis and emphysema of moderate or marked degree are often present.[6] The close approximation of these two reactions in adjacent alveoli is seen infrequently in other conditions to quite the extent in which it has been observed in animals after moderate doses of radiation (Flaskamp; Warren and Gates). When this approximation is marked, it produces a gross picture so distinct as to make it possible to recognize the irradiated portions of the lung with the naked eye (Ludin and Werthemann). Lucarelli described zones of atelectasis in rabbits, but not in dogs, after five exposures to 50 per cent of an erythema dose two days apart. The atelectasis was present on the first day after the completion of the treatment and persisted through the second week, although congestion and edema had subsided. This tendency toward pulmonary atelectasis was accentuated with repeated similar series twenty days apart. More often than not, there is no clear explanation

5. Davis. Tsuzuki. Warren and Gates.
6. Granzow. Lazarus-Barlow. Karlin and Mogilnitzky.

for the variation in size of the alveoli, although plugs of mucus, increased tone of smooth muscle of bronchioles or damaged elastica of alveoli might be considered as a cause.

There has been less opportunity to observe the very early stages of the reaction in human subjects. Edema was present in the majority of the 24 cases in which the lungs showed an acute reaction in Warren and Spencer's series, and it has been mentioned in other reports. Hines described edema, desquamation of alveolar epithelium and fibrosis two months after a four month course of moderate doses of radiation.

Atelectasis has been described in many postmortem reports of irradiated lungs (Freid and Goldberg; Rose). It is undoubtedly the cause of the elevation of the diaphragm and of the shifting of the mediastinal structures toward the affected side so frequently seen as a transitory phenomenon. Desjardins mentioned compensatory emphysema as a result of impaired function after considerable irradiation of the lung.

Cytologic Changes.—Minor change in the epithelium of the bronchial tree is usually the first indication of actual damage to the cells.[7] After repeated light or moderate doses of radiation, or a single heavy dose, there is increased production of mucus and desquamation of cells.[8] The cilia may be absent (Warren and Gates). Provided that there is no further injury, the cells return to normal within a few days or weeks. However, heavier doses of radiation cause a more severe reaction which appears after a latent period of weeks and lasts several months (Engelstad, 1934 a). The epithelium may be columnar or cuboidal, single or many layered (Ludin and Werthemann; Warren and Gates). The circumference of a given level of a bronchiole often shows normal epithelium, mucus-laden columnar cells and piled-up squamous cells. As is usual in radiation change, injured abnormal cells may be side by side with normal cells. Keratinization and calcification of the epithelial cells may be present. In the greatest degree of anaplasia, usually occurring weeks or months after heavy doses of radiation, the cells are very large and syncytial in character (Engelstad, 1934 a; Warren and Gates). Atypical mitotic figures may be present (Ludin and Werthemann; Warren and Gates). Less commonly, the bronchial epithelium forms cords and masses of adenoid-like structures which extend through the muscularis mucosae and suggest autonomous growth (Granzow). Such proliferation is not unlike the reaction seen sometimes in chronic bronchitis and bronchopneumonia, or after severe damage to bronchial

7. In the literature the bronchial and bronchiolar epithelium are not usually distinguished. Warren and Gates found the proliferative and anaplastic changes largely confined to the bronchiolar epithelium in animals.

8. Warren and Gates. Wohlauer. Ludin and Werthemann. Engelstad, 1934 a. Lucarelli.

mucosa from chemical irritants (Winternitz and others); but the more anaplastic changes are seen only after radiation injury (Warren and Gates).

The sequence of the reactions may be made clearer by more detailed sketches of animal experiments. Lucarelli noted desquamation of the epithelial cells of the bronchi of rabbits after three series of exposures to 50 per cent of an erythema dose, each series consisting of five exposures two days apart and each series being twenty to thirty days after the preceding one. Engelstad (1934 a) found more marked changes following larger doses. In rabbits, four hours after 1,700 r had been given in one exposure, the bronchial epithelium secreted mucus somewhat more actively and became swollen and partly desquamated. These disturbances persisted for two or three days and then became less, or completely subsided. After something over two weeks, irregular forms of bronchial epithelium appeared together with increased production of mucus. A similar but more striking alteration in epithelium was seen one to four hours after 4,500 r had been given in one exposure to the thorax and was observed in other animals two months after 9,000 r had been given in three exposures forty-eight hours apart. Warren and Gates found marked anaplasia of bronchiolar epithelium two days after the following treatment: 4,500 r to the thorax in eleven doses of 300 to 600 r each (nine doses in one month; two doses a month later, two days before death).

The effect of gamma rays from 5 Gm. of radium on different species is similar to that produced by roentgen rays. Short exposures stimulate secretion and produce some degenerative changes, while longer exposures cause active proliferation (Lazarus-Barlow).

Abnormalities of bronchial epithelium have been given scant attention in the study of radiation pneumonitis in man. The more striking grades of anaplasia have not been described, although minor abnormalities of the secretion of mucus and metaplasia have been mentioned cursorily (Bauer and Schraer; Voegt). Bronchiectasis, a prominent feature in some human lungs, may be the effect of direct radiation injury to bronchi or a result of secondary infection (Bauer and Schraer; Freid and Goldberg).

The relative sensitivity of the tracheal and bronchial mucosa can only be surmised. Hypertrophy, slight anaplasia of tracheal epithelium and degeneration of mucous glands have been described in patients weeks or months after heavy doses of radiation. The mucosa of the trachea is said to be less sensitive than that of the esophagus (Engelstad, 1934 b).

Before discussing the cytologic changes of the alveoli induced by radiation, a brief inventory of the structure of the alveolar network may not be out of the way. The alveolar walls consist of a mesh of reticular

fibers and less numerous elastic fibers through which the capillaries run. In the normal adult lung there are no obvious lining cells, but a distinct rim of low cuboidal, epithelial-like cells becomes visible in certain states: in atelectasis and in chronic inflammation. They are also seen in the newborn. Their resemblance to epithelium is striking (Miller). The concurrent appearance of alveolar macrophages in inflammation has suggested the identity of these two types of cells. Different sources have been ascribed to these macrophages: (1) septal cells or undifferentiated mesenchymal cells of the alveolar walls; (2) the blood stream or the endothelium of the alveolar capillaries; (3) alveolar lining cells.[9]

For the purposes of this review, it is considered that there are two distinct cell units which are readily seen in the lung under certain abnormal conditions and often, but not always, seen together: (1) the alveolar macrophage and (2) the epithelial cell as observed in a continuous epithelial lining. The effect of radiation on these two types of cells will be considered.

Increased cellularity of the alveolar walls is nearly always present following irradiation of the lung, sufficient to cause marked congestion and edema. This may be due in part to swelling and increased prominence of capillary endothelium but has been attributed to swelling or proliferation of the so-called septal cells (Warren and Gates). Later, alveolar macrophages are numerous in the spaces, and the number varies more or less directly with the intensity of the radiation. They follow fairly closely the other elements of the reaction, becoming more pronounced after two to three months and later disappearing (Engelstad, 1934 a). In rabbits, after heavy doses of radiation (7,040 to 9,000 r in three exposures at forty-eight hour intervals), they may be atypical in appearance, varying in size and shape, with the nuclear chromatin becoming more distinct. Giant cells are formed from coalescence of the macrophages, and degeneration and calcification may be prominent (Engelstad, 1934 a).

The epithelial lining cells also become prominent after moderate or heavy doses of radiation. Some degree of swelling, exfoliation, vacuolation and degeneration, followed by regeneration, have been described in animals by most observers.[10] On the other hand, Wohlauer thought the alveolar epithelium must be extremely refractory to roentgen rays, since it was unchanged in guinea pigs two to eleven days after 7 erythema doses without filter.

It is curious that the most striking degree of anaplasia of alveolar epithelium is seen in man rather than in animals; the reverse may be said of the reaction of bronchial epithelium (Warren and Gates). This

9. Mallory. Maximow and Bloom. Permar.

10. Granzow. Warren and Gates. Ludin and Werthemann. Karlin and Mogilnitzky.

has not been recognized generally. The most graphic description is that given by Warren and Gates and applies to both animals and human beings. First, there is an increase in size of the cells, which maintain their normal relation to the wall, thus producing a fairly uniform lining of alveolar cells, such as occurs in chronic inflammation from many causes.

> . . . From this step there is progression to many and varied forms, the cells becoming often extremely hypertrophic and bizarre and not unlike the giant cells seen in some tumors. The latter forms we have never seen in other than irradiated lungs. As the cells take on these extraordinary shapes, they tend to separate from the basement membrane. At times, tenuous cytoplasmic processes maintain the continuity of the distorted cells with one another or with the alveolar wall. The cell size generally increases with the degree of distortion of outline, sometimes to ten or more times the original size. Often there is no change in the texture or the staining property of the cytoplasm, even though the cell as a whole is markedly distorted. There is often a slight tendency toward basophilic staining; the texture is homogeneous, without evidence of necrobiosis, and the cell boundary is usually clearly defined, though often without a distinct cell membrane. There is no evidence of phagocyte activity. Vacuolation is rarely noted.
>
> As the cell increases in size, the nucleus becomes larger and rather more vesicular, with marked clumping of the chromatin and the presence of one or more large nucleoli. These nucleoli may rival those of regenerating hepatic cells or of the cells of certain types of carcinoma. As a rule, the nucleus remains single and maintains an ovoid contour, but some cells have several nuclei and others strangely irregular ones. Mitotic figures are not seen in these large cells, although normal alveolar cells in animals and occasionally those in man may be seen in mitosis (Warren and Gates).

Similar, though less striking changes have been noted after the administration of radioactive phosphorus to animals (Warren and Gates).

Consolidation, which is seen frequently after irradiation of the lungs, cannot be clearly linked as a direct effect.[11] It occurs in animals after moderate or heavy doses of radiation but does not usually follow repeated light doses given over several months, or a single subepidermicidal exposure.[12] Very heavy doses of radiation may cause necrosis and aseptic consolidation, but secondary infection soon intervenes. Bronchopneumonia is probably the more common form of consolidation and is undoubtedly due to a combination of factors, as lowered resistance of lung tissue, focal atelectasis and loss of ciliated cells. There is a striking chronicity of the more severe grades of inflammatory reaction which recalls the course of chronic radiation ulcers of the skin.

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11. Granzow. Warren and Gates. Engelstad, 1934 a.

12. Tsuzuki. Zweig. Davis. Karlin and Mogilnitzky. Lazarus-Barlow. Ludin and Werthemann. Warthin and Pohle.

Variation in severity of reaction, so characteristic of radiation effect, is particularly striking in cases of pneumonia. Some animals may succumb to pneumonia soon after a dose of radiation which causes only a slight inflammatory reaction in other animals (twenty-five hours after 1,150 r, or ten hours after 4,300 r) (Engelstad, 1934 a). Following 9,000 r (in three exposures forty-eight hours apart), 1 animal died fourteen days later with extensive bronchopneumonia, 2 animals died two months later of confluent bronchopneumonia, and 1 animal died after five months, showing even more severe changes, the lungs being full of cavities separated by dense fibrous tissue. Severe necrosis of the lung was present two to three weeks after 15,000 to 21,000 r given in five or seven exposures to the thorax and, as a result, the animals died in from one to eighteen days (Engelstad, 1934 a). Healing takes place through resolution or organization.

The role of pneumonia as a radiation effect in man is not clear. After therapeutic use of radiation, pneumonia is seldom diagnosed, although the clinical symptoms are often consistent with a low grade infection, and the type of fibrosis found post mortem suggests in many instances, healed pneumonia. Bronchopneumonia and bronchiectasis have been reported post mortem.[13] Myers and McIntosh have reported cases which suggest an increased susceptibility to infection after exposure to radiation. Rajewsky found pneumonia and fibrosis in a case of radium poisoning.

The only feature which can be considered distinctive of radiation effect from these descriptions is the anaplasia of the epithelium of bronchi or alveoli. Recently, Warren and Spencer described the hyaline membrane which when combined with anaplastic epithelium they believe to be pathognomonic for radiation pneumonitis in man. They found it in 22 of 29 of their human cases. Warren and Gates gave a more complete discussion of the membrane in the human subject but failed to find it consistently in animals. Schainer and Krombach attached no specific significance to the hyaline membrane which they found in a case of radiation pneumonitis, nor did Belt in Doenecke's case of radium poisoning and pneumonitis. With these exceptions, the few observations in the literature which one might construe as referring to hyaline membrane are too vague for one to be sure what the author had in mind.[14]

The term "hyaline membrane" refers to dense material staining like hyalin which lies so close to the alveolar wall that at times it appears to be formed by degenerative changes of the wall itself. It is from 1 to 20 or more microns thick, with relatively smooth edges, and is usually acellular. This type of membrane was first described as characteristic of influenza, by Wolbach, and has since been described in other acute

13. Landau. Downs. Freid and Goldberg.
14. Jacobsen. Freid and Goldberg. Granzow. Evans and Leucutia.

and sometimes chronic infections of the lung (Brannon and Goodpasture), but it is extraordinarily rare. Radiation pneumonitis and influenza-pneumonia are the only two conditions in which a hyaline membrane occurs commonly. It is associated with emphysema and atelectasis and has been attributed to the effect of forceful inspiration on fluid in alveoli. It has been further assumed that the fluid contains unusual constituents and for some reason is not quickly absorbed. The membrane probably does not become organized, although fibroblasts may be seen occasionally at the periphery (Warren and Gates).

Supporting Tissue of the Lung and Fibrosis.—In the late stages of radiation reaction it becomes very difficult to know precisely where radiation effect stops and infection begins, especially with reference to the supporting tissues. Few early degenerative changes in connective tissue and elastica have been described. Degeneration of the collagen of alveolar ducts has been seen in dog and shoat, but not accompanied by fibroblastic proliferation (Warren and Gates). Granzow emphasized "degenerative swelling of periarterial connective tissue along with edema" as an early change in guinea pigs. Slight fibrosis of the submucosa of bronchioles was seen in rabbits which had received 4,800 r (Warren and Gates). There is no clear significance in Lucarelli's observation of "intense infiltration of young fibroblastic cells" as well as eosinophils in alveolar septums a day after a course of moderate doses of radiation given over an interval of one hundred and ten days to rabbits and dogs. Perivascular and peribronchial fibrosis are frequently described as a radiation effect in animals.[15] More extensive fibrosis is seen months after heavy doses of radiation, sometimes with extensive necrosis (Engelstad, 1934 a). Granzow suggested that fibrosis may be due to functional changes of the lung as a result of obstructed bronchioles, atelectasis, emphysema and impairment of blood supply.

Postmortem examinations have shown (Warren and Spencer) conclusively that the term "fibrosis" is used indiscriminately and often incorrectly by roentgenologists in connection with certain roentgenographic appearances of the lungs following the use of roentgen radiation and, rarely, that of radium (Adair; Lee and Pack). The role of fibrosis as repair of damaged connective tissue per se is no clearer in human lungs than in the lungs of animals. Postirradiation pulmonary fibrosis rarely is observed in human lungs free from metastatic disease or infection (Downs). It has been described after small doses repeated during a few months as well as after heavy doses (Evans and Leucutia).

Fibrosis has been described as an outstanding feature in most of the autopsy reports.[16] In 29 human lungs showing radiation reaction, it was found impossible to determine the cause of the fibrosis (Warren

15. Karlin and Mogilnitzky. Granzow. Engelstad, 1934 a. Tsuzuki.

16. Freid and Goldberg. Jacobsen. Ruggles. Nathanson. Bauer and Schraer. Fike.

and Spencer). Kalbfleisch described pulmonary fibrosis in a case of radium poisoning.

Following heavy irradiation of dogs and shoats, Weigert's elastic tissue stain demonstrates a marked increase in deep blue or black fibers in the alveolar walls. After 12,300 r to the thorax of a shoat (200 kilovolts in divided doses over four months), these fibers, because of clumping and thickening, form a striking contrast to similar fibers in the normal lung (Warren and Gates). However, Ludin and Werthemann described elastic fibers of rats' and rabbits' lungs diminished or altogether absent after exposure to radiation, and Karlin and Mogilnitzky likewise observed a loss of elastic tissue eight months after three exposures of 2 erythema doses at monthly intervals. Engelstad (1934 a) stained lungs of his animals for elastic tissue. He described "spreading" of the elastic fibrils as due to inflammatory cell infiltration or to fibrosis (Engelstad, 1934 a). The lungs of animals to which 1,060 r had been delivered showed no change in elastic fibers at any of the weekly or monthly intervals of examination (Engelstad, 1934 a).

An increase in the elastic fibers has been described in irradiated lungs without being conclusively attributed to radiation (Warren and Gates). Kalbfleisch found increased and thickened elastic fibers in human lungs showing fibrosis, hypertrophy of smooth muscle and pneumonia as a result of radium poisoning. Voegt described clumping and fragmentation of elastic fibers in radiation pneumonitis.

The smooth muscle of the bronchioles has been described by Granzow as being severely damaged, showing nuclear and protoplasmic degeneration and under some circumstances fragmentation. This degree of change has not been seen by most authors, although some have described swelling of smooth muscle bronchioles after moderate protracted irradiation and heavy irradiation of the lungs of rabbits, dogs and shoats (Warren and Gates).

Voegt described atrophy and fibrosis of bronchial muscle and atrophy of mucous glands of bronchi with radiation pneumonitis in man.

Changes in bronchial cartilages are rarely described in animals or man (Granzow). Engelstad (1934 a) mentioned "clear degeneration of bronchial cartilages" five months after the administration of 9,000 r in three exposures at forty-eight hour intervals. There were also extensive necrosis of the lung and marked metaplasia of bronchial epithelium.

Pleura.—There is no indisputable evidence that the pleura is particularly sensitive to radiation. On the contrary, there is some reason for thinking that most pleural fibrosis is the result of infection rather than of injury by radiation. In the animal experiments in which pleural inflammatory reaction or fibrosis has been observed, there has also been extensive inflammatory reaction in the lungs. Moderate and heavy doses of radiation delivered over a long period to shoats, dogs, rabbits and rats, or heavy doses given in divided form over a short interval

of a few days to rabbits, do not produce significant pleural change (Warren and Gates), though there is ample evidence of the effect of these doses in the parenchyma. From observations on animals, Flaskamp considered the pleura as highly resistant to radiation injury.

Granzow included hydrothorax as a primary effect of radium on the lungs of guinea pigs. This is independent of inflammatory reaction or cardiac influences.

The term "radiation pleuropneumonitis" as used by the roentgenologists indicates a preeminent role for the pleura. This emphasis probably grew out of the early observations on irradiated tuberculous lungs. Although pleural fibrosis often has been emphasized (Voegt; Freid and Goldberg), little attention has been paid to the cases in which extensive radiation reaction was observed without effect on the pleura (Bergonié and Teissier). A case reported by Rose in 1923 is of much interest in this connection. Radiation was given over a period of four years. At death the intercostal muscles were largely replaced by fibrous tissue and there was marked atrophy of the ribs. The lung showed extensive fibrosis, but the pleura was normal except for one dense apical adhesion. Similarly, acute pleuritis and pleural effusions have not been conclusively linked with irradiation of the human chest.

Blood Vessels.—Edema of the walls of arteries and veins is often marked after slight to moderate irradiation of these vessels in rats, rabbits and guinea pigs (Warren and Gates). In many animals endothelial swelling may be seen in large and medium arteries and veins after moderate or heavy doses of radiation and after prolonged exposure to radium (Warren and Gates; Lazarus-Barlow). Proliferation of endothelial cells has not been described after treatment with roentgen rays. Although Pappenheim and Plesch described endothelial injury after intravenous and oral administration of thorium dioxide, it is doubtful that this effect was due to radiant energy. Marked degenerative intimal and medial change in arteries, present several months after delivery of heavy doses of radiation to rabbits, was thought to be a radiation effect and possibly the cause of thrombosis and extensive necrosis of the parenchyma (Engelstad, 1934 a). Medial fibrosis has been described with peribronchial and perivascular fibrosis after heavy irradiation of the thoraxes of rabbits and dogs (Davis) and human beings (Evans and Leucutia). Thickening of the walls of vessels may be due to an increase in bulk from swelling of collagen, which is a well established radiation effect. Marked sclerosis of vessels seen in animals should not be ascribed necessarily to radiation (Ludin and Werthemann).

Vascular changes in irradiated human lungs are of doubtful etiology (Freid and Goldberg; Bauer and Schraer). All types of vascular change are seen in irradiated human lungs, but vascular changes are also com-

mon in normal lungs at middle age and particularly when infection and metastases are present. Coarsening and fraying of the elastica are noted only with higher doses of radiation in some animals and in human lungs.[17]

COMMENT

The lung should be classed as a moderately sensitive structure, since an erythema dose delivered to the skin appears to have a slight transient effect, and the equivalent of such a dose delivered to the lung has a definite, though again a transient, effect. Permanent damage to the lung, however, usually occurs only after much heavier doses of radiation than are customary for therapy. The condition of the lung at the time of exposure to radiation is an important determinant of the reaction. There are two chief dangers in irradiation of the lung: (1) The signs and symptoms may be misinterpreted with the result that treatment is continued; (2) the resistance of the irradiated lung to infection is probably lowered.

Both postmortem and experimental data have proved that the roentgenologic pictures and clinical symptoms usually represent purely transitory changes, which are generally harmless.

Radiation pneumonitis may be divided into three main stages: mild, marked, late. The mild reaction consists of congestion, edema, lymphectasia, sometimes a slight inflammatory cell infiltration and very slight changes in the bronchiolar and alveolar epithelium, such as increased secretion of mucus, loss of cilia, and exfoliation. The reaction may be considered as marked when there is obvious injury or active regeneration of epithelial elements and the presence of a well defined hyaline membrane. In the late reaction, thickening of alveolar walls, patchy atelectasis and vascular change are outstanding, but the other, more acute change may be present also. Fibrosis, though often present, has not been established as a radiation effect independent of intercurrent infection.

Functional studies on the lungs following their irradiation are inconclusive (Stephens and Florey; Swann).

The observations on the effects of irradiation delivered to the thorax suggest comparisons of the tissues exposed with respect to susceptibility. It is probable that the lung parenchyma as such is more resistant to radiation injury than lymphocytes[18] and the gastrointestinal mucosa (Engelstad, 1934 a; Warren and Whipple) but is more susceptible than cardiac muscle, striated muscle, cartilage, bone and probably smooth muscle (Granzow).

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17. Voegt. Warren and Gates. Bauer and Schraer.

18. Since collections of lymphocytes are not integral constituents of human lungs, the effect of radiation on such structures in certain animals has been omitted.

BIBLIOGRAPHY

Adair, F. E.: Am. J. Roentgenol. **35**:359, 1936.

Bauer, J. T., and Schraer, P. H.: Am. J. Path. **16**:657, 1940.

Belt, T. H.: Frankfurt. Ztschr. f. Path. **42**:170, 1931.

Bergonié, J., and Teissier: Arch. d'électric. méd. **6**:334, 1898.

Brannon, D., and Goodpasture, E. W.: Arch. Int. Med. **43**:739, 1924.

Davis, K. S.: Radiology **3**:301, 1924.

Desjardins, A. U.: Am. J. Roentgenol. **16**:444, 1926.

Doenecke, F.: Frankfurt. Ztschr. f. Path. **42**:161, 1931.

Downs, E. E.: Am. J. Roentgenol. **36**:61, 1936.

Engelstad, R. B.: (*a*) Acta radiol., 1934, supp. 19, p. 1; (*b*) **15**:608, 1934; (*c*) **18**:32, 1937.

Evans, W. A., and Leucutia, T.: Am. J. Roentgenol. **13**:203, 1925.

Ewing, J.: Am. J. Path. **16**:659, 1940.

Fike, R. H.: Am. J. Roentgenol. **27**:509, 1932.

Flaskamp, W.: Ueber Roentgenschäden und Schäden durch Radioaktiven Substanzen, in Meyer, H.: Sonderbände zur Strahlentherapie, Berlin, Urban & Schwarzenberg, 1930, vol. 12, p. 147.

Freid, J. R., and Goldberg, H.: Am. J. Roentgenol. **43**:877, 1940.

Gorke, H., and Töppich, G.: Beitr. z. Klin. d. Tuberk. **53**:132, 1922.

Granzow, J.: Arch. f. Gynäk. **151**:612, 1932.

Groover, T. A; Christie, A. C., and Merritt, E. A: South. M. J. **15**:440, 1922; Am. J. Roentgenol. **10**:471, 1923.

——Christie, A. C.; Merritt, E. A., and Coe, F. O.: South. M. J. **20**:153, 1927.

Hines, L. E.: J. A. M. A. **79**:720, 1922.

Hsieh, C. K., and Kimm, H. T.: Am. J. Roentgenol. **37**:802, 1937.

Jacobsen, V. C.: Am. J. Roentgenol. **44**:235, 1940.

von Kalbfleisch, H. H.: Samml. v. Vergiftungsf. **8**:27, 1937.

Kaplan, I. I., and Bell, D.: Am. J. Roentgenol. **39**:387, 1938.

Karlin, M. I., and Mogilnitzky, B. N.: Frankfurt. Ztschr. f. Path. **43**:434, 1932.

Küpferle, L.: Fortschr. a. d. Geb. d. Röntgenstrahlen **21**:85, 1914.

——and Bacmeister, A.: Deutsche med. Wchnschr. **39**:1581, 1913; **42**:96, 1916.

Landau, W.: Ztschr. f. Tuberk. **65**:212, 1932.

Lazarus-Barlow, W. S., in Medical Uses of Radium: Studies of the Effects of Gamma Rays from a Large Quantity of Radium, Medical Research Council, Special Report Series, no. 62, London, His Majesty's Stationery Office, 1922, p. 33.

Lee, B. J., and Pack, G. T.: Acta radiol. **12**:416, 1931.

Lucarelli, G.: Tumori **9**:85, 1935.

Ludin, M., and Werthemann, A.: Strahlentherapie **38**:684, 1930.

McIntosh, H. C.: Radiology **23**:558, 1934.

——and Spitz, S.: Am. J. Roentgenol. **41**:605, 1939.

Mallory, F. B.: The Principles of Pathologic Histology, Philadelphia, W. B. Saunders Company, 1914, p. 37.

Maximow, A. A., and Bloom, W.: A Textbook of Histology, Philadelphia, W. B. Saunders Company, 1938.

Miller, W. S.: The Lung, Springfield, Ill., Charles C. Thomas, Publisher, 1937.

Myers, R. E.: South. M. J. **31**:275, 1938.

Nathanson, L.: New York State J. Med. **37**:1751, 1937.

Pappenheim, A., and Plesch, J.: Ztschr. f. exper. Path. u. Therap. **12**:95, 1913.

Permar, H. H.: J. M. Research **42**:9, 1920.
Rajewsky, B.: Radiology **32**:57, 1939.
Rose, C. B.: J. A. M. A. **80**:1750, 1923.
Ruggles, H. E.: Am. J. Roentgenol. **36**:366, 1936.
Schainer, E., and Krombach, E.: Strahlentherapie **64**:267, 1939.
Stephens, J. G., and Florey, H.: Brit. J. Radiol. **32**:159, 1927.
Swann, M. B. R.: Brit. J. Radiol. **29**:195, 1924.
Tsuzuki, M.: Am. J. Roentgenol. **16**:134, 1926.
Voegt, H.: Virchows Arch. f. path. Anat. **302**:468, 1938.
Warren, S., and Gates, O.: Arch. Path. **30**:440, 1940.
——and Spencer, J.: Am. J. Roentgenol. **43**:682, 1940.
Warren, S. L., and Whipple, G. H.: J. Exper. Med. **35**:187, 1922.
Warthin, A. S., and Pohle, E. A.: Arch. Int. Med. **43**:15, 1929.
Winternitz, M. D.; Smith, G. H., and McNamara, F. P.: J. Exper. Med. **32**: 205, 1920.
Wintz, H.: (*a*) Am. J. Roentgenol. **10**:140, 1923; (*b*) Radiology **1**:74, 1923.
Wohlauer, F.: Deutsche med. Wchnschr. **35**:1704, 1909.
Wolbach, S. B.: Bull. Johns Hopkins Hosp. **30**:104, 1919.
Zweig, H. G.: Strahlentherapie **43**:201, 1932.

Forensic Medicine

NOTES ON THE IDENTIFICATION OF SEMINAL STAINS BY MEANS OF THE FLORENCE REACTION

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The use of the Florence test as a preliminary means of identifying stains suspected to be seminal is well known. A positive reaction is noted on the formation of brown rhomboid crystals which exhibit parallel extinction between crossed Nichol prisms. These crystals are unstable, dissolving in the menstruum in a short period (about thirty minutes). The Florence reaction is extremely sensitive, being more so in my experience than the trinitrophenol test (Barberio test) or the modifications thereof.

The method is as follows: A 0.5 cm. square of cloth is cut from the suspected stain. This is soaked in 0.3 cc. of a 1: 1,000 solution of aerosol OT 85 per cent [1] in water for one hour on a microscopic slide, the slide being covered with a Petri dish to prevent evaporation. The cloth is teased and the fluid wrung out with forceps; 0.1 cc. of the extract is placed on a separate slide and 1 drop of the Florence reagent [2] added. The slide is examined for the presence of the typical crystals. The original extract is stained with methylene blue, and spermatozoa or parts thereof are identified in the wet mount.

The usual garment submitted for examination is filthy, the suspected stain often being contaminated with blood, purulent material, mucus, epithelia, feces and various occupational dusts. While in the majority of instances well formed complete spermatozoa, or their heads (in badly handled specimens), have been easily identified, on several occasions when spermatozoa were not identified it was possible to obtain strongly positive Florence reactions. On four occasions on which there was reason to believe that the stain was exposed to moisture or prevented from drying rapidly, spermatozoa in good condition were observed being slowly destroyed by bacteria; subsequent continuous microscopic observation revealed the head, body and tail to be slowly and completely disin-

From the Crime Detection Laboratory, Department of State Police, University of Oregon Medical School.

1. Aerosol OT (American Cyanide & Chemical Corporation) is the dioctyl ester of sodium sulfosuccinate.

2. The Florence reagent consists of potassium iodide, 5 per cent, and iodine, 8 per cent, in aqueous solution.

tegrated. The solution of sperm, in which no sperm could be identified, continued to give a strongly positive Florence reaction. On three occasions seminal stains which originally contained spermatozoa have been reexamined, areas of cloth contiguous with the original area of examination being utilized; the second examination showed a strongly positive Florence reaction but no identifiable spermatozoa.

While the Florence reagent will give gross amorphous precipitates with aqueous extracts of tissue, feces, lipoids (from liver and kidney) and dried milk, the typical crystalline form has not been observed with these, or with old lecithin, dried egg yolk, dried egg white, nasal and vaginal mucus, serum, sweat, gonorrheal pus, wine, whisky, beer or ice cream residues. Male urine containing spermatozoa gave a positive reaction, but voided specimens in which spermatozoa were absent did not.

SUMMARY

Human spermatozoa on dirty clothing may be disintegrated by bacterial action. The material may give a positive Florence reaction, although spermatozoa cannot be identified as such. This phenomenon may explain some "false positive" Florence reactions.

Stains suspected of being seminal stains should be examined as soon as possible, as the bacterial disintegration may make identification of spermatozoa impossible.

In the medicolegal examination of seminal stains, the Florence reaction alone is insufficient for diagnosis, and spermatozoa must be identified as such.

Notes and News

Appointments.—In the school of medicine of the University of Utah, Louis P. Gebhardt Jr. and Robert E. Hoyt have been appointed associate and assistant professor, respectively, of bacteriology and pathology.

Israel Davidsohn, pathologist at the Mount Sinai Hospital, Chicago, has become editor in chief of the *American Journal of Clinical Pathology* in place of R. A. Kilduff, who has entered military service.

Deaths.—The death of Ludwig Aschoff, professor of pathologic anatomy in the University of Freiburg, Breisgau, Germany, at the age of 75, is announced in *Nature.*

Awards.—The Cleveland medal of the New York City Cancer Committee has been awarded to Elise L'Espérance, a founder of the Strang Cancer Prevention Clinic of the New York Infirmary for Women and Children, "for outstanding contributions to cancer control work."

The Caldwell Medal for distinction in cancer research of the American Roentgen Ray Society has been awarded to Cornelius P. Rhoads, director of the Memorial Hospital for the Treatment of Cancer and Allied Diseases, New York.

Society News.—The Society of American Bacteriologists will hold its annual meeting at the Deschler-Wallick Hotel, Columbus, Ohio, Dec. 28, 29 and 30, 1942.

Book Reviews

The Dynamic State of Body Constituents. Rudolf Schoenheimer. Harvard University Monograph in Medicine and Public Health Number 3. Pp. xiv + 81, with 16 tables, 6 figures, 17 formulas; a bibliography and general references and an index. Price $1.75. Cambridge, Mass.: Harvard University Press, 1942.

This small monograph consists of three lectures delivered by the author at the Harvard Medical School in October 1941. In these lectures, without going into highly technical details as to methods and procedures, he summarized the results and significance of seven years' work on the use of isotopes in the study of intermediary metabolism. The three lectures, now chapters of the book, are respectively entitled:

I. The Reactions of the Body Fats Investigated with Deuterium

II. The State of the Body Proteins

III. The Role of Structural Elements in the Formation of Excretory Products

It has been customary to compare the metabolic system to a combustion engine, in which the food or fuel enters into a fixed system to be converted into energy and waste products. This analogy has been modified by adding that the biologic machine suffers wear and tear, which must also be repaired from the food. The author's conclusion, however, is that not only the fuel but also the structural materials of the machine itself are in a constant state of biochemical flux. The adult living organism, therefore, might better be compared to a military regiment. The latter has a well defined, highly organized structure, and its size fluctuates only within narrow limits. Yet the individuals of which it is composed are constantly changing, as men join, are promoted, are transferred or die. In this analogy the recruits represent the food; transfer, retirement and death correspond to excretion.

While this volume is of primary concern to biochemists and physiologists, all biologists and physicians who would keep abreast of the times might profitably attempt to relate this new concept of living matter to their own particular special fields of interest.

Spontaneous and Experimental Leukaemia in Animals. Julius Engelbreth-Holm, M.D., director of the Cancer Research Laboratory of the Danish Anti-Cancer League; chief pathologist of the Finsen Institute and Radium Station of Copenhagen. Pp. xvii and 237, with 44 figures. Price 15 shillings. Edinburgh and London: Oliver and Boyd, 1942.

This monograph was prepared under the Lady Tata Memorial Trust, and translated from Danish by Mr. C. L. Heel, to coordinate the discoveries in leukemia in animals. It contains parts which deal with spontaneous leukemia in animals, transmission experiments, experimentally produced leukemia and the role of heredity. Finally, in a part on the nature of animal leukemia the conclusions are reached that this process is neoplastic and that it differs in no essential respect from human leukemia. The close relationship if not essential identity of leukemia, subleukemia (or aleukemic leukemia) and lymphosarcoma is emphasized throughout.

This book is a critical, scholarly, readable and fairly comprehensive review of the subjects mentioned. It should be valuable to those who have not kept abreast of the progress in this field, as well as to research workers and to those who have dealt primarily with human leukemia. The illustrations are good, and the bibliography is valuable. It is an excellent companion book to Forkner's "Leukemia and Allied Disorders," which deals primarily with human leukemia.

Books Received

THE PATHOLOGY OF TRAUMA. Alan Richards Moritz, M.D., professor of legal medicine, Harvard Medical School; lecturer in legal medicine, Tufts College Medical School; lecturer in legal medicine, Boston University School of Medicine; pathologist, Massachusetts State Department of Public Safety; consulting pathologist, Massachusetts State Department of Mental Health; associate medical examiner of Suffolk County. Pp. 386 with 117 illustrations. Price $6. Philadelphia: Lea & Febiger, 1942.

STUDIES FROM THE OTHO S. A. SPRAGUE MEMORIAL INSTITUTE, COLLECTED REPRINTS. Volume 25. Chicago: University of Chicago Press, 1940-1941.

THE CARE OF THE AGED. Malford W. Thewlis, M.D., attending specialist in general medicine, United States Public Health Hospitals, New York; attending physician, South County Hospital, Wakefield, R. I.; special consultant, Rhode Island Department of Public Health. Fourth edition, thoroughly revised. Pp. 589, with 35 figures and 15 charts. Price $7. St. Louis: C. V. Mosby Company, 1942.

CENTRAL AUTONOMIC REGULATIONS IN HEALTH AND DISEASE WITH SPECIAL REFERENCE TO THE HYPOTHALAMUS. Heymen R. Miller, M.D., associate attending physician, Montefiore Hospital, New York; Introduction by John F. Fulton, M.D., M.A., D.Phil., (Oxon.) Sterling professor of physiology, Yale University. Pp. 430 with 61 figures. Price $5.50. New York: Grune & Stratton, 1942.

STUDIES OF THE INSTITUTUM DIVI THOMAE. Volume 3, no. 1. Pp. 222. Cincinnati: Institutum Divi Thomae of the Athenaeum of Ohio, 1941.

DE L'ÉPITHÉLIOMA BASOCELLULAIRE SUPERFICIEL. ÉTUDES HISTOLOGIQUES DE L'ARCHITECTURE DE L'ÉPITHÉLIOMA EN COUPES HORIZONTALES EN SÉRIE. Arve Madsen. Pp. 160. Oslo: A. W. Brøggers Boktrykkeri A/S, 1941.

MEDICAL RESEARCH COUNCIL, SPECIAL REPORT SERIES No. 245. Report of the Committee on Bed-Bug Infestation, 1935-1940. Pp. 64. Price 30 cents. London: His Majesty's Stationery Office (British Library of Information, New York), 1942.

ATLAS OF DENTAL AND ORAL PATHOLOGY, PREPARED AT THE ARMY MEDICAL MUSEUM, OFFICE OF THE SURGEON GENERAL, U. S. ARMY, FROM MATERIAL IN THE REGISTRY OF DENTAL AND ORAL PATHOLOGY. Joseph L. Bernier, major, Dental Corps, United States Army, pathologist to the Registry; James B. Mann, colonel, Dental Corps, United States Army, former pathologist to the Registry; J. E. Ash, colonel, Medical Corps, United States Army, curator. Second edition, revised. Pp. 178. Price $5. Chicago: The American Dental Association, 212 East Superior Street, 1942.